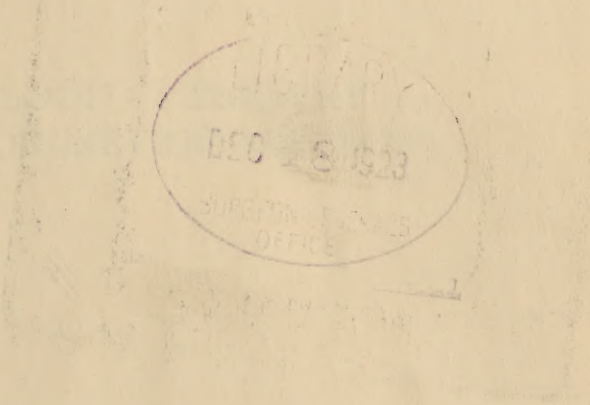
BLOOD CHEMISTRY
COLORIMETRIC METHODS

WILLARD J. STONE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COLORIMETRIC METHODS
For the General Practitioner

WITH
CLINICAL COMMENTS AND
DIETARY SUGGESTIONS

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PREFACE

It is hoped that those interested in the chemistry of the more common blood constituents affected by impaired function or disease will find this compilation useful because of the arrangement of the methods. The methods, which have been slightly modified to meet the demands of the clinical laboratory using relatively small quantities of blood, are dependent entirely upon the exhaustive researches of Folin and Wu, Denis, Lewis, Benedict, Van Slyke, Bloor, Myers, Bailey, and others. Certain clinical comments have been added. The steps to be followed in the determination of total nitrogen in the urine have been modified from the original method of Folin in order to conform to the same strength nitrogen standard used in the determination of non-protein nitrogen in the blood. It has been added for the convenience of those who may be interested in the comparison of blood nitrogen retention, of output nitrogen in the urine and the intake nitrogen from weighed diets. Folin's method for the determination of urinary titratable acidity has been added. For the purpose of ready reference an outline of the essential facts to be determined in the study of impaired kidney function has also been included, together with dietary suggestions covering the treatment of certain disturbed metabolic states.

WILLARD J. STONE.

Pasadena, California
August, 1923.

INTRODUCTION

Discoveries in biological chemistry have stimulated many valuable investigations, which are not utilized as much as they should be in clinical medicine and especially in private practice. This neglect has not been peculiar to chemical methods. Many other diagnostic procedures have been less used than they should be, for similar reasons, the chief of which has been ignorance of the methods. All physicians, however, should be familiar with new methods, while those of recent laboratory training should be able to repeat them and become expert in their use. A frequent excuse for neglect has been the fear that the methods reported were imperfect and would be improved upon or modified. This has been true also of many other means of diagnosis, but should not deter the physician with the investigative turn of mind from such work, since by means of it the results can be compared, the sources of error and the fallacies recognized, and improvements adopted.

The time required for the methods, rather than the cost of apparatus or reagents, has been an important factor for the busy physician. A well-equipped small laboratory will, however, be able to do quite as satisfactory work as the large scale performance of tests in public laboratories, but with the added advantage that the physician may modify and improve his own methods and check his own work. An important reason for the wider application of chemical methods in diagnosis depends upon the importance of the findings as a guide to the condition of the body with normal or disturbed function. Many who have had such tests applied have been discouraged because diagnoses, especially names of diseases, have not been furnished. But this ignores the fact that in practice the name of the disease is not so important as accurate knowledge of the physiology of the patient. Also that the changes which may occur from day to day are of more importance than the results of a

single test. So in the case of many chemical methods the course of the various changes is essential just as a temperature curve is more useful than an isolated observation of the body heat. While some of the present methods may in the light of experience be abandoned, it is certain that many others will become as necessary as the simpler clinical examinations.

Dr. Stone has given the essential details of the most valuable clinical methods of biochemistry, methods that have been extensively used by himself and others. Those already familiar with such work will find the book useful for reference, while those who have been discouraged by the mass of detail given in more exhaustive textbooks will find it a clear and accurate guide. The use of such methods of clinical study will add not only interest but greater accuracy to the work of the physician and enable him with satisfaction to take part in the general advance of clinical knowledge. The large field of clinical chemistry is open to those who are interested in giving to their patients advice founded upon facts rather than fads or fancies. It should also be recalled that many discoveries in the field of medicine have come from small laboratories, a reason which should give further stimulation to the more general adoption of such investigative methods of work.

GEORGE DOCK.

Pasadena, California
August, 1923.

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CHAPTER I

Suggestions for Blood Chemistry Work

1. For blood chemistry work the fasting state is desirable, since practically all of the established normal values have been obtained from blood specimens taken twelve to fourteen hours after the ingestion of food. If the blood specimen is taken during the period of greatest absorption from the gastro-intestinal tract, within a period of three or four hours after a meal, the figures for non-protein nitrogen, urea, and sugar may be considerably increased. for which due allowance should be made in interpreting the results. As to the method of taking the blood specimen the following plan has been found satisfactory. About 6 c.c. of blood are drawn from one of the veins at the bend of the elbow by means of a dry glass syringe. One and one-half c.c. are expelled into a small tube for the routine Wassermann test while the balance is expelled into another tube containing 2 or 3 drops of a 20 per cent potassium oxalate solution to prevent clotting. This tube is inverted several times to mix thoroughly the oxalate with the blood.

2. It has been found most satisfactory to make the tests for non-protein nitrogen, urea, creatinin, uric acid, and sugar upon the blood filtrate as described in the methods of Folin and Wu.

3. The picric acid method of Lewis and Benedict (or its modification by Myers and Bailey) for blood sugar may give higher readings in many specimens of blood, primarily because of the absorption of creatinin by the picric acid. While not criticizing the usefulness of this test, it is believed that more uniform results are obtained by following a procedure such as that offered by the method of Folin and Wu. For verification

of blood sugar in any given case the Lewis and Benedict picric acid method is recommended.

4. Chemically pure sodium tungstate and picric acid are essential. (See notes in the text.) Some preparations of sodium tungstate are not satisfactory because of an excess of carbonate. Sodium tungstate, Primrose Brand, or Merck's c.p. sodium tungstate are satisfactory. The Nessler's solution should be carefully prepared.

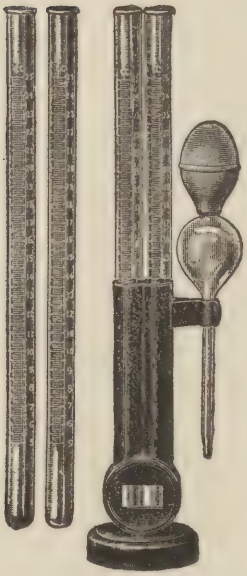

FIG. 1. Myers colorimeter.

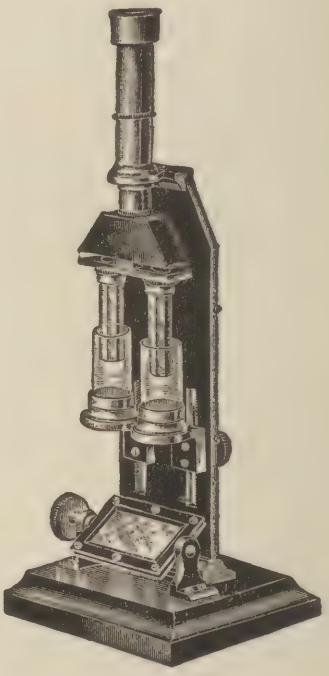

FIG. 2. Duboscq colorimeter.

5. For standard sugar solutions, chemically pure dextrogucose (Pfanstiehl) may be recommended. The standard nitrogen, uric acid, and creatinin solutions can be secured from any dealer in scientific supplies by those who do not have facilities for making their own solutions.

6. Discrepancies in the calibration of glassware, especially pipettes, burettes, graduates, and volumetric flasks, should be noted when equipping the laboratory. It is best to have on hand for comparison a few utensils the accuracy of which has been confirmed by the Bureau of Standards, Washington.

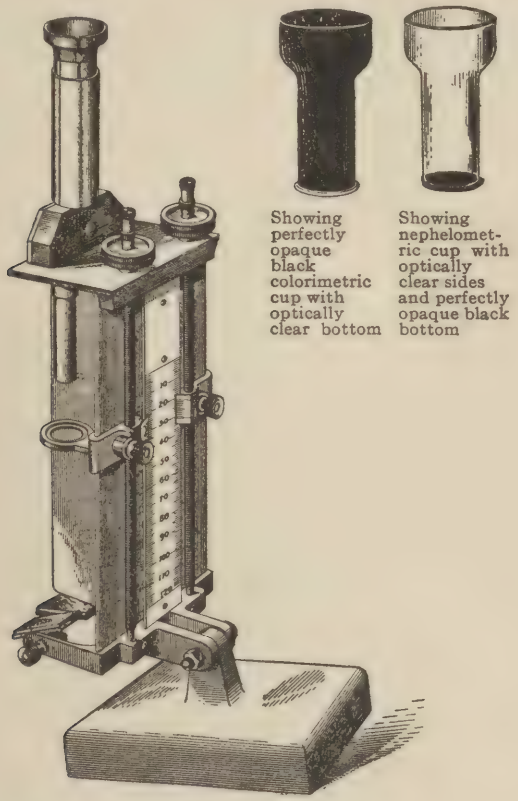

FIG. 3. Kober colorimeter.

7. It is wise to calibrate frequently the colorimeter scale in order to determine that approximately identical readings are secured, using for comparison the standard solutions used in the tests. If the Kober colorimeter is used, the mirrors should be carefully adjusted to reflect the same amount of light into

the cups, which should be filled about two-thirds full of the solutions. If discrepancies occur, the scale should be adjusted so that the readings coincide.

8. The blood pipettes and blood-sugar tubes of Folin, with bulb and constriction, as well as Pyrex ignition test-tubes, can be obtained from any dealer in scientific supplies.

9. The choice of the colorimeter will depend upon the individual. The Duboscq is now made in this country, as well as the Kober and the Bock-Benedict models.

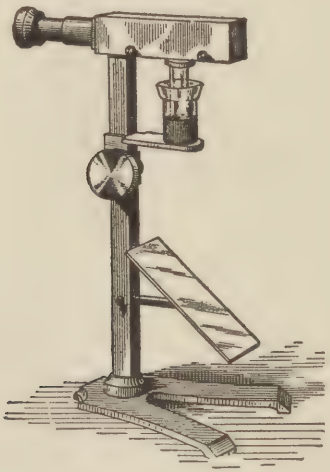

FIG. 4. Bock-Benedict colorimeter.

Formula for Calculation in Colorimeter Work

The following formula serves as the basis for computations in which different quantities of unknown and varying strengths of standard solutions are used:

$$\frac{\text{Standard Reading}}{\text{Unknown Reading}} \times \frac{\text{Strength of Standard in Mg.}}{\text{Volume of Standard as Compared with Unknown}} = \text{Mg. in Amount of Blood or Urine Used.}$$

THE NORMAL BLOOD CONSTITUENTS OF CLINICAL IMPORTANCE

	PER 100 C.C. OF BLOOD
Urea	12.0 – 15.0 mg.
Uric acid	1.5 – 3.0 mg.
Preformed creatinin	1.5 – 2.0 mg.
Total creatinin (creatin plus creatinin)	4.0 – 6.0 mg.
Amino-acid nitrogen	6.0 – 8.0 mg.
Ammonia nitrogen	0.1 – 0.2 mg.
Total non-protein nitrogen (the incoagulable nitrogen)	25.0 – 35.0 mg.
Sugar	80.0 –110.0 mg.
Cholesterol	160.0 –200.0 mg.
Chlorides (as NaCl)	600.0 –650.0 mg.

CHAPTER II

Preparation of Protein-Free Blood Filtrate Used in Determination of Non-Protein Nitrogen, Urea, Uric Acid, Preformed Creatinin, Total Creatinin, Sugar and Chlorides (Method of Folin and Wu[1])

I. Solutions Used in the Preparation of Protein-Free Blood Filtrate

1. Sodium tungstate, c. p. ..... 20.0 gm.
   Distilled water to ..... 200.0 c.c.
2. *⅔ Normal Sulphuric Acid*
   Sulphuric acid ..... 35.0 gm. by weight
   Distilled water to ..... 1000.0 c.c.

   This solution, while approximately correct, should be checked up by titration, since in the quantities used it is intended to be equivalent to the sodium content of the tungstate.

II. Transfer exactly 4 c.c. of the oxalated blood to 100 c.c. flask. Avoid an excess of oxalate because of interference with uric acid precipitation. Add 28 c.c. of distilled water and shake to lake the blood. Add 4 c.c. of 10 per cent sodium tungstate solution and mix. Add from graduated burette, *slowly and with constant shaking, 4 c.c. of ⅔ normal sulphuric acid.* Close flask with rubber stopper and shake. The shaking should produce but little foaming. Let stand for five minutes. The color should change from red to brown. If color does not change the coagulation is incomplete, usually because of too much oxalate. In such an event add 10 per cent sulphuric acid, one drop at a time, and shake after each drop until there is no foaming and the brown coloration has occurred. It is important to avoid

[1] *J. Biol. Chem.*, 1919, xxxviii, 81.

adding any excess of sulphuric acid beyond the amount required to secure thorough protein precipitation since an excess may also precipitate the uric acid and interfere with its subsequent determination.

Pour the mixture slowly on filter paper and cover with watch glass. The filtrate should be clear.[1] If filtrate is to be kept for a day or two add a few drops of xylol or toluol.

Ten c.c. of the blood filtrate equals 1 *c.c. of blood.*

Non-Protein Nitrogen

I. Solutions Used in the Determination of Non-Protein Nitrogen in the Blood

1. *Acid Phosphoric-Sulphuric Digestion Mixture*

Copper sulphate (5 per cent solution)	50.0 c.c.
Acid phosphoric (85 per cent)	300.0 c.c.
Acid sulphuric, c.p. (ammonia-free)	100.0 c.c.
Distilled water	450.0 c.c.

Keep well stoppered to prevent absorption of ammonia from the air.

2. *Stock Nessler's Solution*

KI	15.0 gm.
Iodine	11.0 gm.

in 100 c.c. flask; add

Water	10.0 c.c.
Metallic mercury	14.5 gm.

Shake flask vigorously, using rubber cork, for seven to ten minutes until dissolved iodine has nearly all disappeared. The solution becomes quite hot. When the red iodine solution has begun to pale in color, cool in running water and continue shaking until red color of iodine has been replaced by greenish color of the double iodide (about ten minutes). Separate solution from surplus mercury by decanting and washing with distilled water. Dilute solution with washings to 200 c.c.

[1] The blood filtrate, if the correct strength of acid has been used, should be only slightly acid to Congo-red paper.

3. *Preparation of Nessler's Reagent from Stock Nessler's Solution*

Put into 500 c.c. bottle:

10 per cent solution of NaOH	350.0 c.c.
Stock Nessler's solution	75.0 c.c.
Distilled water	75.0 c.c.

(In the preparation of the 10 per cent solution of NaOH used in Nessler's reagent it has been found much more satisfactory to make the solution accurately by weight instead of by volume.)

1 c.c. contains approximately:

Hg metallic	0.1 gm.
KI	0.01 gm.
Iodine	0.001 gm.
NaOH	0.07 gm.

4. *Standard Nitrogen Solution*

Ammonium sulphate (highest purity)	0.9432 gm.
Distilled water	1000.0 c.c.

1 c.c. = 0.2 mg. nitrogen

II. Preparation of Unknown Solution

To 2.5 c.c. of filtrate, in large Pyrex ignition test-tube, add 0.5 c.c. of the acid phosphoric-sulphuric digestion mixture. Boil gently over micro-burner until water has been nearly evaporated. Cover tube with watch glass and continue boiling gently for about two minutes. Dense fumes from the acid will rise in the tube. The solution will turn dark brown and upon heating slowly will soon turn nearly colorless. Allow tube to cool. Wash contents of tube carefully with 5 to 7 c.c. of distilled water into a 25 c.c. volumetric flask. This completes the preparation of the unknown solution with the exception of the addition of Nessler's reagent. (See below.)

III. Preparation of Standard Solution

The standard usually required is about 0.2 mg. of nitrogen per 100 c.c. Place with measuring pipette 1 c.c. of standard

nitrogen solution (which contains 0.2 mg. per c.c.) in 50 c.c. volumetric flask, add 1 c.c. of the phosphoric-sulphuric acid digestion mixture to balance acid in the unknown, and then add about 15 c.c. of distilled water.

IV. Final Step

Add 8 to 10 c.c. of Nessler's reagent slowly to flask containing the unknown, and when full development of color has occurred, fill to 25 c.c. mark with distilled water. Insert clean rubber stopper in flask and mix. If solution is turbid, centrifuge small portion before comparing with standard.

Add 12 to 15 c.c. of Nessler's reagent slowly to standard solution in volumetric flask and when full development of color has occurred add distilled water to 50 c.c. mark. Insert clean rubber stopper and mix. (*The Nessler's reagent should be added as nearly simultaneously as possible to unknown and standard solutions.*)

Compare unknown in colorimeter with standard set at 20 mm.

V. Calculation

The equivalent of 0.25 c.c. of blood was used; the standard solution contained 0.2 mg. nitrogen; the volume of standard was twice the volume of the unknown solution.

The calculation will be as follows, R indicating the reading of the unknown:

$$\frac{20}{R} \times \frac{0.2}{2} \text{ mg.} = \text{mg. non-protein nitrogen in 0.25 c.c. blood}$$

or

$$\frac{800}{R} = \text{mg. non-protein nitrogen in 100 c.c. blood.}$$

The average of many analyses of normal blood specimens has been between 25 and 35 mg. of non-protein nitrogen per 100 c.c. of blood.

Determination of Blood Urea

(Method of Folin)

I. Solutions Used

1. *Buffer Mixture to Activate the Urease Solution*
   Sodium pyrophosphate (U. S. P.)..... 140.0 gm.
   Glacial phosphoric acid..... 20.0 gm.
2. *Urease Solution*
   Wash about 3 gm. of permutit in a flask with 2 per cent acetic acid, decant and repeat the process twice with water; add 5 gm. of Jack bean meal to 16 c.c. of 95 per cent alcohol and 84 c.c. of water. Shake for ten minutes, filter and collect the filtrate in 3 or 4 different small bottles. This solution will keep for one week at ordinary room temperature, but may be preserved for four to six weeks in an ice box. Direct sunlight exposure of the solution should be avoided.
3. $\frac{N}{20}$ *Hydrochloric Acid*
   Concentrated HCl ..... 1.0 c.c.
   Distilled water to ..... 200.0 c.c.
   (This solution is approximately correct.)
4. *Saturated Borax Solution*
5. *Paraffin Oil*
6. *Standard Nitrogen Solution*
   Ammonium sulphate (highest purity). 0.9432 gm.
   Distilled water..... 1000.0 c.c.
   1 c.c. = 0.2 mg. nitrogen.

II. Preparation of Unknown Solution

To 2.5 c.c. of blood filtrate in a large Pyrex test tube (which must previously have been rinsed with nitric acid and then with water if it has contained Nessler's solution), add one drop of buffer mixture and 0.5 c.c. of urease solution. Immerse

the tube in warm water (40°–55°C.) for five minutes or let stand at room temperature for fifteen minutes.

To collect the ammonia formed from the urea without using a condenser, a test-tube with perforated rubber stopper containing a curved glass tube is used for distillation as in the accompanying illustration. The distillate is collected in a graduated 25 c.c. perforated rubber-stoppered receiving tube containing 1 c.c. of $\frac{N}{20}$ hydrochloric acid solution.

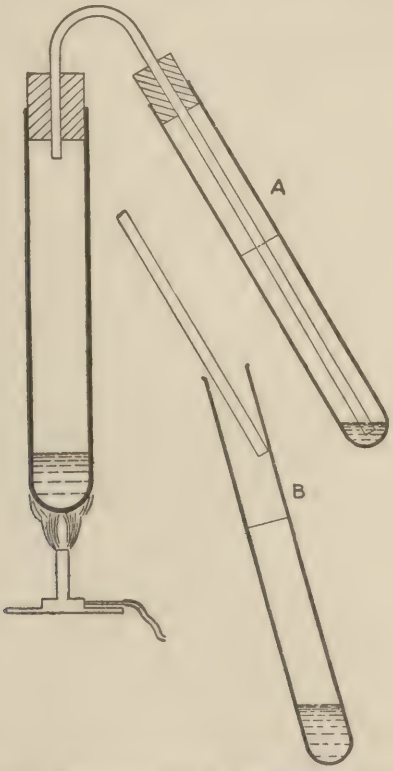

FIG. 5. A at beginning, B toward end of distillation. From Folin's Manual of Biological Chemistry. Courtesy of D. Appleton and Company.

Add to the blood filtrate a dry pebble, a drop or two of paraffin oil and 1 c.c. of the borax solution. Boil, using a small flame at a uniform rate, for about four minutes. The boiling

should not be so brisk that the emission of steam occurs from the receiving tube before three minutes. At the end of four minutes disconnect the receiving tube from the rubber stopper, let it rest in a slanting position and continue the distillation for a minute longer. Rinse out the delivery tube with a little water, and after cooling the distillate with running water add the washings to it and bring the volume to 10 c.c. by adding sufficient distilled water.

III. Preparation of Standard Solution

Transfer 1 c.c. of standard nitrogen solution to a 50 c.c. volumetric flask, dilute to about 40 c.c., add about 7.5 c.c. of Nessler's solution, rotate flask until Nesslerization is complete and add water to the 50 c.c. mark.

IV. Final Step

Add 1.5 c.c. of Nessler's solution to the unknown and after Nesslerization is complete dilute to the 12.5 c.c. mark with water.

V. Calculation

The equivalent of 0.25 c.c. blood was used; the standard solution contained 0.2 mg. nitrogen; the volume of standard was 50 c.c., while the volume of unknown was 12.5 c.c.

Compare unknown in colorimeter with standard set at 20 mm.

The calculation will be as follows, R indicating the reading of the unknown:

$$\frac{20}{R} \times \frac{0.2}{4} = \text{mg. in } 0.25 \text{ c.c. blood,}$$

or

$$\frac{400}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

VI. Clinical Comments on Non-Protein Nitrogen and Urea in the Blood

Marked increase of non-protein nitrogen (urea constituting 70–80 per cent) in the blood may be expected in partial or com-

plete suppression of kidney function, whether acute or chronic. If complete, the condition is that usually described as uremia. It is also increased in such conditions as prostatic hypertrophy producing urinary retention and bilateral ureter compression or obstruction. The increase is especially marked in the type of nephritis brought about by poisoning with the heavy metals such as lead, arsenic and mercury. In many patients with arterial hypertension due to contracted arterioles (high diastolic blood-pressure) with its associated cardiorenal symptoms, there may be little evidence of abnormal nitrogen retention as long as the kidneys are permeable, fluids freely excreted and a low protein diet compatible with their excretory capabilities is being followed. When, however, as a result of infection, overindulgence, or poisoning, the capacity of the kidneys for the elimination of waste nitrogen is overtaxed, an acute exacerbation of a preexisting nephritis, giving perhaps few symptoms, may occur, with rapid increase in the quantities of retained nitrogen in the blood.

In early kidney damage or impaired function the non-protein nitrogen of the blood is usually moderately increased, i.e., to 40–50 mg. This increase is largely made up of urea, nitrogen and uric acid. During the process of digestion urea is formed in the liver from ammonia resulting from the breaking down of the protein food constituents into amino-acids. Urea is therefore of exogenous origin. The uric acid results from the action of enzymes or other glandular constituents upon amino and oxypurins. It is usually considered to be partly of endogenous and partly exogenous origin. In the condition described as acute nephritis the retention of non-protein nitrogen is higher, often reaching 150 mg. per 100 c.c. of blood.

In the chronic types of diffuse or interstitial nephritis the retention may be less marked, especially if the patient is living within the functional capacity of his kidneys. In such chronic types the non-protein nitrogen retention will usually be found to vary from 50 to 100 mg. per 100 c.c. of blood. The types of chronic nephritis associated with severe anemia are particularly subject to blood nitrogen retention. This type of anemia,

which seems to bear no constant relation to edema, is often characterized by a high hemoglobin index, approaching 1 or 1+, while the changes in the red cells usually expected in severe anemias, such as stippling and irregular shapes and sizes, are lacking. As has been suggested by Berg,[1] this hyperchromatic type of anemia probably results from profound changes in the activity of the blood-forming organs. It is not improbable that prolonged nitrogen retention and associated acidosis may bear some etiologic relationship to it.

In the condition described as parenchymatous nephritis or "nephrosis," nitrogen retention is, except in the terminal stages of the disease, not markedly increased. In the terminal stage abnormal nitrogen retention usually occurs. In eclampsia there is usually a moderate increase in the non-protein nitrogen of the blood. This increase results from a marked retention of uric acid rather than retention of urea. The evidence points to a certain degree of impaired kidney function, not only because of the uric acid retention, but also because the threshold of sugar elimination is increased, which results in a moderate hyperglycemia. Eclampsia should not be described as a condition due to "uremia" according to knowledge of the subject now available.

In many chronic conditions, such as arteriosclerosis or malignancy, abnormal blood nitrogen retention may develop as evidence of the impaired function and associated changes involving the kidneys. Such retention, if persistent and if uninfluenced by diet, undoubtedly influences the prognosis. In some severe acute infections, such as diphtheria and pneumonia, blood nitrogen retention may occur. This seems to be especially marked if dehydration and acidosis is an associated condition. In acute intestinal obstruction the retention of non-protein nitrogen or urea may reach three or four times the normal figures.

In prostatic obstruction, blood chemistry studies are important in that knowledge of the associated kidney changes gives indication as to the most suitable time for any contem-

[1] Berg. *Am. J. M. Sc.*, 1922, clxiv, 88.

plated operation for relief. If the non-protein nitrogen figure does not exceed 35 mg. per 100 c.c., the uric acid 3 mg. and creatinin 2 mg. per 100 c.c. of blood, the patient may be considered a reasonably good operative risk. If these figures are appreciably exceeded the evidence of kidney damage should indicate caution. Institution of measures designed to relieve gradually the hydrostatic back-pressure upon the kidneys, such as preliminary bladder drainage, are of great value in restoring impaired kidney function to these patients. The retention of non-protein nitrogen, uric acid and creatinin in the blood may rapidly diminish under such treatment, the extent of the reduction depending, of course, upon the extent of the previous damage.

CHAPTER III

Uric Acid

(New method of Folin[1])

I. Solutions Used in the Determination of Blood Uric Acid

1. *Silver Lactate Solution* (10 *per cent*)

   Dissolve 50 gm. of silver lactate in 350 c.c. of warm water and add a mixture consisting of 50 c.c. of 85 per cent lactic acid and 50 c.c. of 10 per cent sodium hydroxide. Add water to 500 c.c. The sediment present should be allowed to settle and only the clear supernatant solution used in the test.

2. *Acidified Sodium Chloride Solution*

   Concentrated HCl..... 1.0 c.c.
   10 per cent sodium chloride sol..... 100.0 c.c.

3. *Lithium Sulphate Solution* (20 *per cent*)

   Dissolve 20 gm. of powdered lithium sulphate (Baker & Adamson's) in 80 c.c. of cold water. Dilute to volume of 100 c.c. and filter.

4. 15 *Per Cent* (*approx.*) *Solution of Sodium Cyanide*

   Prepare enough to last three months, since this solution is believed to improve with age. Use white solid sodium cyanide not discolored or decomposed by exposure to air. Weigh out from 100 to 200 gm. of cyanide, transfer to beaker, add 6.7 c.c. of 0.1 normal sodium hydroxide solution for each gram of cyanide taken and stir until all has dissolved. The solution is opalescent. Transfer to bottle and keep at least two weeks before using. The 0.1 normal sodium hydroxide

[1] *J. Biol. Chem.*, October, 1922, liv, 153.

was added to prevent decomposition with discoloration. Ammoniacal decomposition of the cyanide sooner or later destroys its efficiency, since maximum color development is retarded and turbidity is produced. This can be prevented by covering the stock bottle with a beaker instead of using a cork stopper, or by boiling off the ammonia and then diluting to the original volume. Because of its toxicity it should, in performing the tests, be measured from a burette.

To test for the blank due to impurity of the cyanide, transfer 5 c.c. of water, 2 drops of lithium sulphate solution and 2 c.c. of the 15 per cent cyanide solution to a test-tube. Add 1 c.c. of the uric acid reagent (given below) and let stand two minutes. The solution should remain colorless. Heat in boiling water for one and a half minutes. Some color will develop. To determine whether this color will materially affect the uric acid values in a test, repeat the above with two graduated test-tubes and with standard uric acid solution, 5 c.c. in one and 3 c.c. plus 2 c.c. of water in the other. Dilute to volume after heating and compare the colors. If the cyanide is suitable the weaker solution will give the theoretical reading, 33.5 mm., when the stronger solution is placed in colorimeter at 20 mm.

5. *Stock Uric Acid-Formaldehyde Solution*

Transfer exactly 100 mg. uric acid[1] to a funnel on a 100 c.c. volumetric flask. Dissolve 45 to 50 mg. of lithium carbonate in 15 c.c. of water by heating to about 60°C. until all the carbonate has been dissolved. With the hot carbonate solution rinse the uric acid on the funnel into its flask and shake. The uric acid will promptly dissolve. Cool flask under running water by shaking and add 40 to 50 c.c. of water. Then add 2.5 c.c. of 40 per cent formaldehyde and, after shaking to insure thorough mixing, acidify by the addition of 0.3 c.c. of glacial acetic acid. Shake to remove most of the

[1] Uric acid (Kahlbaum) is a satisfactory preparation.

carbonic acid, dilute to 100 c.c. and mix. This stock solution should be kept tightly corked in a dark bottle.

1 c.c. = 1 mg. uric acid.

6. *Standard Uric Acid Solution*

Place 1 c.c. of the stock solution, with a graduated pipette, in a 250 c.c. volumetric flask, add 125 c.c. of water and 10 c.c. of the ⅔ normal sulphuric acid used in blood protein precipitation, then add 1 c.c. of 40 per cent formaldehyde, dilute to 250 c.c. and shake to mix.

1 c.c. = 0.004 mg. uric acid.

7. *Uric Acid Reagent of Folin and Denis*

In flask place

Water	75.0 c.c.
Sodium tungstate	10.0 gm.
Phosphoric acid (85 per cent)	8.0 c.c.

Partly close flask with funnel and small watch glass and boil gently for two hours, then dilute with water to 100 c.c.

II. The Test (Short Method)[1]

Have ready for use a wide-mouthed beaker containing boiling water.

Place 5 c.c. of the blood filtrate and 2 c.c. of water in a test-tube graduated at 25 c.c.

Place in a similar tube 5 c.c. of the standard uric acid solution and 2 c.c. of water.

Add 2 or 3 drops of 20 per cent lithium sulphate solution to each. The lithium sulphate solution is added to prevent precipitate formation in the presence of potassium oxalate used for anticlotting purposes. It may be necessary at times to add 4 drops of the lithium sulphate solution to prevent precipitation.

[1] The quantity of lithium sulphate necessary to prevent precipitation, together with the precipitation which may result after boiling for eighty seconds, has been found to be such a source of trouble in some specimens of blood that the more complete "silver lactate method" given on page 19 is preferred.

From the burette add 2 c.c. of 15 per cent sodium cyanide solution to each tube.

With a graduated pipette add 1 c.c. of the uric acid reagent to each tube, mix and let stand two minutes.

At the end of two minutes transfer both tubes to the boiling water for eighty seconds. Longer heating may cause precipitation.

Cool the tubes, add water to the 25 c.c. mark and mix by inverting the tubes.

III. Calculation

Read the standard against itself set at 20 mm. If the two scales do not coincide, adjust to secure correct reading.

Equal volumes of unknown and standard were used.

The equivalent of 0.5 c.c. of blood was used.

The standard contained 0.02 mg. uric acid.

With R indicating the reading of unknown the calculation will be:

$$\frac{20}{R} \times 0.02 \text{ mg.} = \text{mg. uric acid in } 0.5 \text{ c.c. blood,}$$

or

$$\frac{80}{R} = \text{mg. uric acid in 100 c.c. blood.}$$

This short method may give values from 0.1 to 0.2 mg. higher than those obtained by the following method, which employs more certain recovery by means of silver lactate precipitation.

IV. The Test (Silver Lactate Method)

Have ready for use a beaker containing boiling water.

Place 5 c.c. of the blood filtrate in a centrifuge tube, add 7 c.c. of the 10 per cent silver lactate solution. Mix and centrifuge. All the uric acid is contained in the precipitate. Decant the supernatant fluid as completely as possible.

Add 1 c.c. of the acidified sodium chloride solution to the precipitate, stir thoroughly with a glass rod, add 4 c.c. of water and, after stirring, centrifuge.

Pour the supernatant solution into a test-tube graduated at 25 c.c.

Place 5 c.c. of standard uric acid solution (containing 0.004 mg. per c.c.) in a test-tube graduated at 25 c.c.

To the contents of the unknown and to the standard tube, add 0.2 c.c. of 20 per cent solution of lithium sulphate, 2 c.c. of the 15 per cent sodium cyanide solution from burette, and 1 c.c. of the uric acid reagent. Shake each tube and let stand for two minutes.

Heat the two tubes in boiling water for eighty seconds, cool, add water in each tube to the 25 c.c. mark and compare in colorimeter as in the short method.

V. Calculation

Read the standard against itself set at 20 mm. If the two scales do not coincide, adjust to secure correct reading.

Equal volumes of unknown and standard were used.

The equivalent of 0.5 c.c. of blood was used.

The standard contained 0.02 mg. uric acid.

With R indicating the reading of unknown the calculation will be:

$$\frac{20}{R} \times 0.02 \text{ mg.} = \text{mg. uric acid in } 0.5 \text{ c.c. blood,}$$

or

$$\frac{80}{R} = \text{mg. uric acid in } 100 \text{ c.c. blood.}$$

Uric acid in the blood normally varies from 1.5 to 3.0 mg. per 100 c.c. The latter figure may be considered a high normal.

Uric Acid

(Benedict's Modification, using the blood filtrate method of Folin and Wu[1])

I. Solutions Used in the Determination of Blood Uric Acid

1. *Arsenic-Phosphotungstic Acid Reagent*[2]

Add 100 grams sodium tungstate, c.p., to 600 c.c. distilled water, and after dissolved add 50 gm. pure arsenic pentoxide, 25 c.c. of phosphoric acid (85 per cent) and 20 c.c. of concentrated hydrochloric acid. Boil the mixture for twenty minutes, cool and dilute to 1000 c.c. (This reagent keeps indefinitely and yields nearly seven times as much color as does the "uric acid reagent" of Folin and Denis. It is scarcely affected by polyphenols in the presence of uric acid.)

2. *5 Per Cent Sodium Cyanide Solution*

Sodium cyanide	12.5 gm.
Concentrated ammonia	0.5 c.c.
Distilled water to	250.0 c.c.

This solution should be freshly prepared once in two months. It should be measured from a burette in performing the test because of its toxicity.

3. *Stock Uric Acid Solution*

Dissolve 2.25 gm. of pure crystals of hydrogen disodium phosphate and 0.25 gm. of dihydrogen sodium phosphate in 75 c.c. of hot distilled water. Filter and make up to 125 c.c. with hot water. Pour this warm clear solution on 50 mg. pure dried uric acid (Kahlbaum) suspended in a few c.c. of water in a 250 c.c. volumetric flask. Agitate until completely dissolved and add at once exactly 0.35 c.c. of glacial acetic acid,

[1] Benedict. *J. Biol. Chem.*, March, 1922, li, 187.

[2] The arsenic pentoxide used in the "arsenic-phosphotungstic acid reagent" is marketed under the name "acid arsenic c.p. powdered."

then add distilled water nearly to the 250 c.c. mark, add 1.5 c.c. of chloroform and finally add distilled water to the 250 c.c. mark. The solution should be freshly prepared every two months. Before weighing it will be best to dry the uric acid at about 100°C. in an oven for an hour or two.

1 c.c. of the stock solution = 0.2 mg. uric acid.

4. *Standard Uric Acid Solution*

Measure 10 c.c. of the stock uric acid solution (containing 2 mg. uric acid) into a 500 c.c. volumetric flask and fill the flask about half full of distilled water, add 25 c.c. of dilute hydrochloric acid (one volume of concentrated acid diluted to ten volumes with distilled water) and dilute the solution to 500 c.c. This standard should be freshly prepared once in two weeks.

1 c.c. = 0.004 mg. uric acid.

II. Preparation of Unknown Solution

Transfer 5 c.c. of the blood filtrate to a graduated test-tube and add 5 c.c. of distilled water. Add 4 c.c. of the 5 per cent sodium cyanide solution.

III. Preparation of Standard Solution

Transfer 5 c.c. of the standard solution to a graduated test-tube and add 5 c.c. of distilled water. Add 4 c.c. of the 5 per cent sodium cyanide solution.

IV. Final Step

Add 1 c.c. of the arsenic-phosphotungstic acid reagent to the tube containing the unknown and to the tube containing the standard solution. Mix by inverting tubes and place them immediately in boiling water for three minutes. Then remove tubes and place in beaker of cool water for three minutes. Compare in colorimeter within five minutes because of tendency to turbidity.

V. Calculation

Equal volumes of unknown and of standard solutions were used.

The standard contained 0.02 mg. An equivalent of 0.5 c.c. of blood was used.

Therefore if S equals the height of standard solution in mm. and R equals the reading of the unknown, $\frac{S}{R} \times 0.02 \times 200$ or $\frac{S \times 4}{R}$ = mg. of uric acid per 100 c.c. of blood.

Note on Benedict's Uric Acid Method. Benedict's method has been found by Brown and Raiziss[1] to give higher readings, due to interfering substances, than the original method of Folin and Wu. In the following table their comparative results are given when the same protein-free filtrate was used for the two methods:

	Uric Acid (Folin and Wu) mg. per 100 c.c.	Uric Acid (Benedict) mg. per 100 c.c.
1	2.2	3.2
2	1.9	2.8
3	1.8	4.7
4	2.6	3.6
5	5.2	6.3
6	3.0	3.9
7	2.0	3.1
8	3.2	4.5
9	4.0	5.8
10	6.2	6.6

VI. Clinical Comments on Uric Acid Retention

Uric acid is probably the first non-protein nitrogen constituent to be retained in abnormal amounts by the blood in early,

[1] Brown, H., and Raiziss, G. W. The estimation of uric acid in blood. *J. Lab. & Clin. M.*, November, 1922, viii, 129.

temporary or permanent damage to the kidneys. A distinctly higher than normal value, above 3 mg. per 100 c.c., if persistent and not remedied by appropriate dietary restrictions and other possible contributing causes, should attract attention to the possibility of kidney impairment. Uric acid is almost invariably high in gout, while the total non-protein nitrogen value may be within normal limits.

In acute gout the blood uric acid not uncommonly reaches 8 to 10 mg. if the patient is not on a low purin diet. In chronic gout, with which condition there is so many times evidence of associated or concomitant kidney impairment, the amount of uric acid commonly retained by the blood reaches 6 to 8 mg. per 100 c.c. This can be decreased in some instances by a low protein-low purin diet, although for many patients with chronic gout the influence of a low-purin diet on the blood uric acid is not marked. The administration of uric acid eliminants, such as cinchophen and tolysin or the salicylates, is many times of service.

In suspected gouty arthritis without tophi, but in which an increased blood uric acid figure is obtained, if the non-protein nitrogen and creatinin are also increased, gout may be excluded and the increase in uric acid be more reasonably ascribed to impaired kidney function. In non-gouty arthritis without kidney impairment the blood uric acid is usually within normal limits. Since uric acid has its origin in the body partly from endogenous and partly from exogenous sources, disturbances of metabolism resulting in retention not uncommonly lead, in those whose elimination is impaired, to muscle pains, stiffness, and headaches. If confirmation of the suspected disturbance is obtained by finding an abnormal retention of uric acid in the blood, the condition, which is usually temporary, may in many instances be relieved by a low-purin diet and the administration of cinchophen or tolysin.

CHAPTER IV

Preformed Creatinin
(Method of Folin and Wu)

I. Solutions Used in the Determination of Preformed Creatinin in the Blood

1. *Alkaline-Picrate Solution*

Saturated (1.2 per cent) picric acid solution	8.5	c.c.
10 per cent sodium hydroxide solution	1.5	c.c.

2. *Stock Creatinin Solution*

Creatinin-zinc chloride	1.61	gm.
Tenth-normal HCl solution	1000.0	c.c.

1 c.c. = 1 mg. creatinin.

3. *Standard Creatinin Solution*

Stock creatinin solution	5.0	c.c.
Tenth normal HCl solution	10.0	c.c.
Distilled water to	100.0	c.c.

Add two or three drops of xylol as a preservative.
1 c.c. = 0.05 mg. creatinin.

II. Preparation of Unknown Solution

To 5 c.c. of blood filtrate in graduated tube add 2.5 c.c. of alkaline-picrate solution. Mix and allow to stand six to eight minutes to develop color.

III. Preparation of Standard Solution

Measure with pipette 0.3 c.c. of standard creatinin solution (containing 0.05 mg. per c.c.) into graduated tube and add water, using same pipette, to 10 c.c. mark. Add with accurate pipette 5 c.c. of alkaline-picrate solution. Mix and let stand

six to eight minutes to develop color. (The standard solution thus prepared contains 0.015 mg. creatinin.)

IV. Calculation

Note that both fields are equal when both cups of colorimeter contain the standard set at 20 mm.

The color comparison between standard and unknown should be made within fifteen minutes from the time the alkaline-picrate was added.

With standard set at 20 mm., R indicating the reading of the unknown solution, the computation will be as follows, since the equivalent of 0.5 c.c. of blood was used:

$$\frac{20}{R} \times \frac{0.015}{2} = \text{mg. in 0.5 c.c. of blood.}$$

or

$$\frac{30}{R} = \text{mg. in 100 c.c. blood.}$$

The average of many analyses has shown the normal amount of preformed creatinin to be about 1.5 mg. per 100 c.c. of blood.

Total Creatinin (Creatin plus Creatinin)

I. Solutions Used

Same as for preformed creatinin determination.

II. Preparation of Unknown Solution

Place 2 c.c. of blood filtrate in 10 c.c. volumetric flask, add 0.5 c.c. of normal hydrochloric acid. Cover mouth of flask

Picric Acid Purity. To test purity of picric acid as used in creatinin and blood sugar determinations, Folin and Doisy (*J. Biol. Chem.*, 1916–17, xxviii, 349) have suggested the following procedure: Add 1 c.c. of 10 per cent NaOH solution to 20 c.c. of a saturated (1.2 per cent) solution of picric acid in water. The color of the alkaline-picrate solution so prepared must not be more than twice as deep a color as that of the saturated picric acid solution. With unusually pure picric acid, the color of the alkaline-picrate solution will not be more than one and one-half times as deep as that of the picric acid solution, i.e., with picric acid solution set at 20 mm. in the colorimeter the alkaline-picrate solution will give a reading of 13 to 14 mm.

with tinfoil and heat in cup of boiling water for twenty minutes. Cool. Add 2 c.c. of freshly prepared alkaline-picrate solution. Allow to stand five minutes and dilute with water to 10 c.c. mark.

III. Preparation of Standard Solution

Place 0.5 c.c. of the creatinin standard solution (containing 0.05 mg. per c.c.) in a 20 c.c. volumetric flask. Add 1 c.c. of normal hydrochloric acid,[1] and 4 c.c. of freshly prepared alkaline-picrate solution. Allow to stand five minutes, then add water to 20 c.c. mark. The standard so prepared contains 0.025 mg. creatinin.

IV. Calculation

Fill both colorimeter cups half full of standard solution as prepared above, and determine whether both fields are equal with tube length set at 20 mm. If both are not alike adjust vernier scale of right-hand tube by thumb-screw (Kober instrument) so that tube length corresponds to left-hand scale.

Adjust mirrors so that reflected light is equal in each field. Empty the right-hand tube and wash. Also wipe solution from plunger. Fill this tube half full of unknown solution and make comparison. R indicates reading of the unknown.

$$\frac{20}{R} \times \frac{0.025}{2} = \text{mg. in 0.2 c.c. blood,}$$

or

$$\frac{125}{R} = \text{mg. in 100 c.c. blood.}$$

The normal value for total creatinin by this method is about 6 mg. per 100 c.c. of blood.

[1] *Normal HCl.* Concentrated HCl (molecular weight 36.46) is approximately ten times a normal solution, therefore 25 c.c. conc. HCl plus distilled water to 250 c.c. constitutes a normal solution; and 25 c.c. of this latter solution plus distilled water to 250 c.c. will make a tenth-normal solution sufficiently accurate for this test.

V. Clinical Comments on Blood Creatinin

Creatinin is considered to be the most easily eliminated non-protein nitrogen constituent of the blood under conditions of normal kidney function. For this reason considerable impairment of kidney function may exist without retention of creatinin beyond normal limits. When extensive impairment of kidney function has occurred, retention of creatinin results. An increase of preformed creatinin in the blood to 4 or 5 mg. per 100 c.c. has therefore great diagnostic and prognostic importance. Its persistence at a high level indicates severe nephritis except in prostatic or bilateral ureteral obstruction or compression. With persistent retention of 5 mg. or more per 100 c.c. of blood, few patients live longer than a few months. In acute retention due to prostatic obstruction the preformed creatinin may reach 10 mg. with recovery when the obstruction is relieved.

Behre and Benedict (*J. Biol. Chem.*, May, 1922) have recently cast doubt upon the presence of creatinin in the blood. They believe that the blood does contain creatin, the source of which is muscle tissue and which represents in the blood a waste product for elimination by the kidneys. During the process of elimination it is converted into creatinin. They believe that it is creatin rather than creatinin which is retained in the blood when the renal function is disturbed. As a matter of correct phraseology involving physiological facts it is important that their work be confirmed. Such confirmation would not particularly change the clinical fact that creatin bodies are retained above the normal limits in the blood, particularly in the chronic forms of nephritis.

CHAPTER V

Blood Sugar

(New Method of Folin and Wu[1])

I. Solutions Used in the Determination of Blood Sugar

1. *Stock Sugar Solution, 10 mg. per c.c.*

Dextrose, c.p.	1.0 gm.
Distilled water to	100.0 c.c.

Add a few drops of xylol to preserve.

2. *Standard Sugar Solution, 0.1 mg. per c.c.*

Stock sugar solution	5.0 c.c.
Distilled water to	500.0 c.c.

Add a few drops of xylol to preserve.

3. *Molybdate-Phosphate Solution*

Molybdic acid (85 per cent) c.p.	17.5 gm.
Sod. tungstate, c.p.	2.5 gm.
10 per cent sod. hydrate sol.	100.0 c.c.
Distilled water	100.0 c.c.

Boil vigorously for twenty to thirty minutes to remove ammonia, cool, dilute to about 175 c.c., add 62.5 c.c. of concentrated (85 per cent) phosphoric acid, then dilute to 250 c.c.

4. *Alkaline Copper Tartrate Solution*

Anhydrous sod. carbonate, c.p.	20.0 gm.
Distilled water	200.0 c.c. in flask;
Add	
Tartaric acid	3.75 gm., and when dissolved
Add	
Crystallized copper sulphate	2.25 gm.

[1] Folin and Wu. *J. Biol. Chem.*, 1920, xli, 367.

Mix and make volume to..... 500.0 c.c.

(This is the same solution as that mentioned in Folin's earlier method.)

II. Preparation of Unknown Solution

Place 2 c.c. of the blood filtrate in a Folin blood-sugar tube graduated at 25 c.c. Add 2 c.c. of alkaline copper tartrate solution. The surface of the mixture should reach the constricted part of the tube. If the bulb is too large for the volume (4 c.c.), not more than 0.5 c.c. of a diluted 1 to 1 alkaline copper tartrate solution may be added. If this does not suffice to bring the contents to the narrow part, the tube should be discarded; likewise, if the bulb is so small that 4 c.c. fill it above the neck, the tube should be discarded.

III. Preparation of Standard Solution

Place in another similar tube 2 c.c. of standard sugar solution equal to 0.2 mg. of dextrose and add 2 c.c. of the alkaline copper tartrate solution.

IV. Final Step

Place the two tubes in a cup of boiling water for six minutes. Then place tubes in a cup of tap water to cool for two or three minutes.

Add to each tube 2 c.c. of the molybdate-phosphate solution which dissolves the cuprous oxide usually within two minutes. When dissolved, dilute the resulting blue solutions in the tubes to the 25 c.c. mark, insert a rubber stopper and invert the tubes to mix. This should be done carefully, since the greater part of the blue color has been formed in the bulb of the tube.

It is important that the unknown and the standard tubes be heated the same length of time, and also that they be approximately the same temperature when the molybdate-phosphate solution is added. In this method reoxidations of the cuprous compounds are excluded, the blank due to blue alkaline copper

tartrate is eliminated, and the error due to so-called phenols in the blood filtrate is removed.

V. Calculation

The standard should be set at 10 mm.

Equal volumes of unknown and standard solutions were used.

The standard contained 0.2 mg. of dextrose.

The equivalent of 0.2 c.c. of blood was used.

The calculation will therefore be as follows, R indicating the reading of the unknown:

$$\frac{10}{R} \times 0.2 \text{ mg.} = \text{mg. in } 0.2 \text{ c.c. blood,}$$

or

$$\frac{1000}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

The average of many analyses of normal blood specimens has been from 80 to 110 mg. of sugar per 100 c.c.

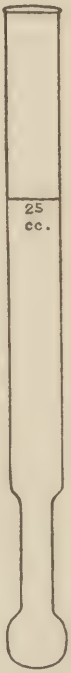

Fig. 6. Folin Blood-Sugar Tube.

Blood Sugar

(Picric Acid Method)

(Modified from the Lewis-Benedict and Myers-Bailey Methods)

I. Solutions Used in the Determination of Blood Sugar by the Picric Acid Method

1. *Hydrochloric Acid, 2.5 per cent Solution*

Acid hydrochloric, conc.	2.5 c.c.
Distilled water to	100.0 c.c.

2. *Picrate-Picric Acid Solution*

Dry powdered picric acid, c.p.	36.0 gm.
Sodium hydroxide, 1 per cent solution	500.0 c.c.
Hot water	400.0 c.c.

Shake until dissolved and when cool add distilled water to 1000 c.c.

3. *Myers-Bailey Picric Acid Sugar Standard*

Dextro-glucose, anhydrous, c.p..... 0.01 gm.
Picric acid, c.p., saturated sol. in water.. 100.0 c.c.
1 c.c. = 0.1 mg. sugar.
(This solution keeps indefinitely.)

4. 20 *Per Cent Sodium Carbonate Solution*

Sodium carbonate, anhydrous, c.p..... 20.0 gm.
Distilled water to..... 100.0 c.c.

II. Preparation of Unknown Solution

Place 1 c.c. of oxalated blood in 15 c.c. tube; place 2 c.c. of 2.5 per cent hydrochloric acid solution in a small graduate and rinse pipette by drawing the solution up in it two or three times to remove blood adhering to wall; add the washings to the blood and shake to lake it.[1] Then add exactly 7 c.c. of picrate-picric acid solution. Insert cork and shake to mix thoroughly. Filter through paper.

Place 3 c.c. of the filtrate (equal to 0.3 c.c. blood) in graduated tube and add 1 c.c. of 20 per cent sodium carbonate solution.

III. Preparation of Standard Solution (Myers and Bailey)

Place in graduated tube 3 c.c. of the Myers-Bailey picric acid sugar standard; add 1 c.c. of 20 per cent sodium carbonate solution. This standard contains 0.3 mg. glucose.

IV. Final Step

Place in cup of boiling water for ten minutes both the unknown and standard tubes. Cool the tubes to room tempera-

[1] Two c.c. of 2.5 per cent solution HCl, when added to 1 c.c. of blood when final mixture equals 10 c.c. will bring the acid dilution to 0.05 normal, which amount Benedict has shown (*J. Biol. Chem.*, 1919, xxxvii, 503) is necessary, for proper precipitation of the proteins, in addition to the picrate-picric acid solution used for that purpose.

Three c.c. of the 2.5 per cent HCl will be necessary if 2 c.c. of blood are used and the final dilution equals 15 c.c.

ture and then add distilled water to each to the 10 c.c. mark. Allow ten minutes for the development of color and then compare in the colorimeter with standard set at 15 mm.

V. Calculation

R indicates the reading of the unknown.

$$\frac{15}{R} \times 0.3 \text{ mg.} = \text{mg. in } 0.3 \text{ c.c. blood,}$$

or

$$\frac{1500}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

VI. Clinical Comments on Blood Sugar

In diabetes occurring in patients with impaired kidney function the non-protein nitrogen constituents of the blood may also be increased in addition to the increase in blood sugar. The threshold of sugar excretion in the urine may also be higher. The renal threshold for sugar excretion, however, varies within quite wide limits. It has been believed that with so-called normal kidneys sugar will appear in the urine when the blood sugar reaches 170 to 175 mg. per 100 c.c. In diabetics of long standing, sugar may be absent in the urine when the blood sugar has reached 250 to 350 mg. per 100 c.c., due to the associated nephritis. In early diabetes the blood will be found to contain from 160 to 200 mg. of sugar per 100 c.c. The threshold of sugar excretion in the urine may be unimpaired when the blood sugar is only moderately increased to 160 to 170 mg. and no sugar may be found in the urine. It is important, however, to recognize in such patients the probability that an early diabetic state exists, regardless of the absence of sugar in the urine, since so much may be accomplished for their benefit by appropriate dietary restrictions.

For individuals with lowered ability to metabolize carbohydrates it is important to determine their capacity by performing the so-called glucose tolerance test. This consists in determining the sugar content of the blood in the fasting state (before breakfast). A urinary specimen is to be taken at this time.

The patient is then given 100 gm. of anhydrous glucose dissolved in 250–300 c.c. of iced water to which the juice of a lemon has been added. Specimens of blood are taken for the determination of sugar at the end of one hour, two hours, and three hours. At the end of each of these periods the patient empties the bladder. Each specimen of urine is examined for sugar, and if found to be present the percentage is estimated in the polariscope or by means of Benedict's quantitative method. The patient should drink about 200 c.c. of water each hour while the test is in progress.

The interpretation of the findings may be summarized as follows: For the normal individual the blood sugar is increased during the first hour after taking the glucose, reaching its maximum at that interval and falling again to normal at the end of two hours. In the pre-diabetic state the rise in blood sugar is most marked at the end of the first hour, but approaches the normal level more slowly at about the end of the three hour interval or longer. In the diabetic state the rise of the blood-sugar level is more slowly reached, the maximum amount being found in the blood at the end of two or three hours. The normal level (equal to the amount of sugar in the blood prior to the test) is reached more slowly, usually at the end of five to eight hours.

In so-called "renal diabetes," a rare condition, the blood sugar may be normal while the urine contains glucose which persists and is unaffected by carbohydrate restrictions. The kidneys are more permeable to sugar excretion than normal, that is, the "threshold" for sugar excretion is below the level of the normal sugar in the blood.

In the condition known as "alimentary glycosuria" sugar may be found in the urine during the period of alimentary absorption after a meal rich in carbohydrates. The sugar will not be found in the urine during the fasting state ten to twelve hours after a meal.

CHAPTER VI

Blood Chlorides

(Method of Whitehorn,[1] using the protein-free filtrate of Folin and Wu)

I. Solutions Used in the Determination of Blood Chlorides

1. *Silver Nitrate Solution*

   Dissolve 4.791 gm. of c.p. silver nitrate in distilled water and add up to the 1000 c.c. mark in volumetric flask. Preserve in dark bottles. 1 c.c. = 1 mg. Cl.

2. *Sulphocyanate Solution*

   This should be prepared volumetrically. Add about 3 gm. of potassium sulphocyanate or 2.5 gm. of ammonium sulphocyanate to 1000 c.c. of distilled water. By titration and dilution the solution should be standardized so that 5 c.c. are equivalent to 5 c.c. of the silver nitrate solution.

3. *Nitric acid*, concentrated, of a specific gravity of 1.42.

4. *Sodium tungstate*, used in the preparation of the protein-free blood filtrate. This should be free from chlorides. To test, mix one volume of sodium tungstate solution with two volumes of concentrated chloride-free nitric acid and filter into a test-tube containing silver nitrate solution. Turbidity indicates contamination with halogen.

II. Method

Accuracy is especially important in measuring the solutions, since slight variations in the amount of chlorides may be of significance. Volumetric flasks are recommended for the 1–10 dilution.

With a pipette place 10 c.c. protein-free filtrate (equivalent to 1 c.c. blood) in a porcelain dish. Add 5 c.c. of the standard

[1] Whitehorn, J. C. *J. Biol. Chem.*, February, 1921, xlv, 449.

silver nitrate solution and stir thoroughly. Then add 5 c.c. of concentrated nitric acid, mix by stirring and let stand five minutes. Then add with a spatula an abundant amount (about 0.3 gm.) of powdered ferric ammonium sulphate as indicator.

Titrate the excess of silver nitrate with the standard sulphocyanate solution until the definite salmon red (not yellow) color of the ferric sulphocyanate persists when stirred for a few seconds.

III. Calculation

Each c.c. of the sulphocyanate solution used in titration is equivalent to 1 c.c. of the silver nitrate solution. The difference between the number of c.c. of silver nitrate solution taken and the excess by titration, i.e., 5 minus the number of c.c. of sulphocyanate solution titrated, will represent the volume which reacted with chloride in the ratio of 1 c.c. to 1 mg. of Cl.

Since 1 c.c. of blood was used, the calculation will be 5 minus the number of c.c. of standard sulphocyanate solution used = mg. of Cl per c.c. of blood. To convert Cl figures into NaCl divide by 0.606. Multiply by 100 to obtain the result per 100 c.c. of blood.

Example: It required by titration 1 c.c. of standard sulphocyanate solution to produce the end reaction. The result would be 5 minus 1 = 4 mg. of Cl per c.c. of blood, or 6.6 mg. sodium chloride per c.c. or 660 mg. per 100 c.c. of blood.

Blood Chlorides

(Method of Rieger,[1] using the protein-free filtrate of Folin and Wu and based upon the principle of Rappleye)

I. Solutions Used in the Determination of Blood Chlorides

1. A sodium tungstate solution prepared as follows to remove chlorides: Prepare a 10 per cent solution of sodium tungstate and after acidifying with an equal volume of concentrated nitric acid filter off the lemon-yellow precipitate. To the fil-

[1] Rieger, J. B. *J. Lab. & Clin. M.*, October, 1920, vi, No. 1.

trate, if clear after the addition of a few more drops of nitric acid, a few drops of silver nitrate test solution are added, which should not be turbid when viewed by transmitted light if free from chlorides. To purify the tungstate solution it is poured into a cylinder containing an equal volume of 50 per cent sulphuric acid. The precipitate is allowed to settle and the supernatant fluid is then siphoned or poured off. The precipitated acid is then washed by decantation until the test for chlorides is no longer given. The precipitate is then dissolved in the requisite amount of 40 per cent sodium hydroxide, using 7 c.c. for each 10 gm. of sodium tungstate taken. The reaction of the resulting solution should be adjusted with dilute sulphuric acid until neutral to litmus. Enough water is then added to make a solution with sp. gr. of 1.15. This is filtered and is then ready for use as a 10 per cent neutral chloride-free solution of sodium tungstate.

2. *Standard Silver Solution*

Silver nitrate crystals	7.2653	gm.
Nitric acid, sp. gr. 1.42	250.0	c.c.
Sat. sol. iron ammonium alum	50.0	c.c.
Distilled water to	1000.0	c.c.

3. *Ammonium Sulphocyanate Solution*

Ammonium sulphocyanate	0.75	gm.
Distilled water	1000.0	c.c.

This should be adjusted by titration so that 25 c.c. equals 5 c.c. of the silver solution.

4. *Sulphuric Acid ⅔ Normal Solution*

Sulphuric acid	35.0	gm. by weight
Distilled water to	1000.0	c.c.

This solution is approximately correct.

II. Method

Place 5 c.c. of the sodium tungstate in a 50 c.c. volumetric flask, add 5 c.c. of oxalated blood and 5 c.c. of ⅔ normal sulphuric acid. The flask is well agitated and allowed to stand for one hour. Distilled water is then added to the 50 c.c. mark,

the flask agitated and the contents filtered. The filtrate should be water white and give no precipitate with an equal volume of nitric acid (absence of tungstate). The presence of tungstate greatly obscures the end point in the succeeding titration.

Twenty c.c. of the filtrate, which represent 2 c.c. of blood, are placed in a 50 c.c. volumetric flask. To this are added 10 c.c. of distilled water and 10 c.c. of the standard silver solution. Distilled water is then added to the 50 c.c. mark. The flask is shaken vigorously to coagulate the silver chloride. The suspension is then filtered. Twenty-five c.c. of the filtrate are then titrated with the ammonium sulphocyanate solution to the appearance of the first brown tinge. The reaction is quite sharp.

III. Calculation

The number of cubic centimeters of sulphocyanate solution used to secure the reaction is subtracted from 25. The difference is then multiplied by 50 to obtain the number of milligrams of sodium chloride per 100 c.c. of whole blood.

Example. It required 12.2 c.c. of sulphocyanate solution by titration to secure the reaction of a brown tinge to the silver chloride filtrate. Subtracting this from 25 would equal 12.8, which, multiplied by 50, would equal 640 or the number of mg. of sodium chloride per 100 c.c. of blood.

IV. Clinical Comments on Blood Chlorides

Under normal conditions the blood contains about 650 mg. chlorides (as sodium chloride) per 100 c.c. In chronic nephritis the content may vary between 450 and 750 mg., depending upon the ability of the kidneys to excrete. In diabetes mellitus and insipidus the chloride content of the blood is decreased because of the diuresis. In edema associated with cardiac and renal disease the chloride concentrations are usually increased because of the absence of diuresis. This is especially true in that form of kidney disease designated as chronic parenchymatous nephritis or "nephrosis." In pneumonia the blood chlorides are relatively low and correspond with the lowered urinary chloride excretion in this disease.

CHAPTER VII

Blood Cholesterol

(Modified Method of Bloor[1])

I. Solutions Used in the Determination of Blood Cholesterol

1. *Cholesterol Stock Solution*
   Cholesterol (Kahlbaum)..... 0.2 gm.
   Chloroform (pure)..... 200.0 c.c.
   1 c.c. = 1 mg. cholesterol.
2. *Cholesterol Standard Solution*
   Cholesterol stock solution..... 10.0 c.c.
   Chloroform (pure)..... 90.0 c.c.
   1 c.c. = 0.1 mg. cholesterol.
3. *Alcohol (redistilled)*
4. *Ether*
5. *Chloroform (dry)*
6. *Acetic anhydride*
7. *Sulphuric acid (concentrated)*

II. Preparation of Unknown Solution

Place 3 c.c. of whole blood slowly (with constant shaking of the flask) in a mixture of 60 c.c. of redistilled alcohol and 20 c.c. of ether in a 100 c.c. graduated flask. Shake thoroughly.

The flask is placed in a water bath on an electric hot plate and the contents carefully raised to boiling. Care should be taken not to overheat, by frequently shaking the flask. After boiling point has been reached, cool flask to room temperature, fill to the 100 c.c. mark with the alcohol-ether mixture, thoroughly mix and filter. (The filtered liquid will keep in a tightly stoppered dark bottle until the next day if necessary before completing the final determination.)

[1] Bloor. *J. Biol. Chem.*, 1916, xxiv, 227; 1917, xxix, 437.

Place 10 c.c. of the alcohol-ether filtrate in a small beaker and evaporate just to dryness on a water bath or electric plate. Care should be used not to heat beyond the point of dryness, as a brownish color is produced, which renders the determination difficult.

The dry residue in the beakers is then extracted with successive small amounts (2 to 3 c.c.) of dry chloroform.[1] The residue and chloroform suspension are brought to a boil on a water bath. Decant each time after boiling to half volume into a 10 c.c. glass-stoppered graduated cylinder or graduated test-tubes. After cooling add chloroform up to 5 c.c. The solution should be colorless. Slight turbidity does not interfere. Add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid.[1] Mix by inverting cylinder several times. The unknown contains the equivalent of 0.3 c.c. blood.

III. Preparation of Standard Solution

Place 5 c.c. of the standard cholesterol solution in a 10 c.c. glass-stoppered graduated cylinder. Add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Mix by inverting cylinder several times. The standard solution which contains 0.5 mg. cholesterol begins to fade in about twenty minutes, so that comparison should be made within an interval of fifteen minutes.

IV. Final Step

Set tubes containing unknown and standard solutions aside at room temperature for about five minutes, after which place in colorimeter cups for comparison with standard set at 15 mm. An average of three or four readings should be taken.

[1] Georgine Luden in her work on Cholesterol (*J. Lab. & Clin. M.*, September, 1919, iv, 719) found that any trace of water in the chloroform interfered with the subsequent color reaction. If the chloroform is kept in a wide glass bottle, into which has been placed a quantity of calcium chloride, any trace of water will be taken up by the latter substance. The chloroform should be filtered before use. Luden has found that adding 0.2 c.c. of concentrated sulphuric acid instead of 0.1 c.c., as generally recommended, produces a tone of green that can be matched more readily without interfering with the cholesterol values.

V. Calculation

$$\frac{15}{R} \times 0.5 \text{ mg.} = \text{mg. in } 0.3 \text{ c.c. blood,}$$

or

$$\frac{2500}{R} = \text{mg. in } 100 \text{ c.c. blood}$$

VI. Clinical Comments on Blood Cholesterol

The origin of blood cholesterol is not definitely known. Some of it arises from exogenous and some from endogenous sources. It is subject to quite wide variations under conditions of disturbed metabolism. The test should not be regarded as a diagnostic test, but rather as a clinical test similar to the determination of hemoglobin or the test for albumin in the urine. The normal amount of cholesterol present in whole blood varies between 160 and 200 mg. per 100 c.c. In the plasma the figure is higher, averaging about 230 mg., while the corpuscle content averages about 200 mg. per 100 c.c.

In mild diabetes the cholesterol content of whole blood is usually increased to 240 to 250 mg. per 100 c.c., while in severe types of the disease the content is increased to 350 to 410 mg. per 100 c.c. The increase of the cholesterol content in diabetes is relatively proportionate to the increase of fat and total fatty acids of the blood in this disease.

In cholelithiasis due to cholesterin concretions, the blood cholesterol is sometimes increased to 280 mg. or more, 950 mg. being mentioned by Hawk. This latter figure may be considered unusual, for many instances of proven cholelithiasis have not shown an increase in blood cholesterol above the range of normal values.

The problems of disturbed cholesterol metabolism are concerned especially with the activity and quantity of bile and pancreatic juice available to convert cholesterol into esters. The adrenals and other glands are also concerned in the process. De Zani's experiments, mentioned by Georgine Luden in her work on cholesterol, have shown the importance of this lipoid

to the cellular integrity of the organism. He fed mice on a cholesterin-free diet. During this time the animals drew upon their reserve deposits of cholesterol present in the body fat, brain, adrenal cortex and the liver, for cholesterol was present in the feces. The mice increased in size and weight, but they all died at the end of seventeen days.

The recent work of Epstein and Lande[1] has called attention to the importance of blood cholesterol studies in connection with protein deficiency and decreased basal metabolism, especially in parenchymatous nephritis (nephrosis). In their observations on 6 such patients the basal metabolism was subnormal and was associated with high blood cholesterol figures. In these patients with large amounts of albumin in the urine and decreased output associated with extensive edema, and who did not improve upon salt-free, Karrell, and carbohydrate diets, an improvement in basal metabolism and decreased edema was noted under a high protein diet together with the administration of thyroid extract. In myxedema, which may be associated with nephrosis, the blood cholesterol may be increased. Under thyroid therapy with improvement in the basal metabolic rate in such cases the blood cholesterol has decreased.

In exophthalmic goiter with marked increase in the basal metabolic rate, as well as in toxic adenomas of the thyroid, the blood cholesterol values are usually lower than normal. The following table arranged from the publication of Epstein and Lande recapitulates their findings:

[1] Epstein, A. A., and Lande, H. The relation of cholesterol and protein deficiency to basal metabolism. *Archiv. Int. Med.*, November, 1922.

BLOOD CHOLESTEROL CONTENT IN RELATION TO METABOLIC RATE

Condition	Number Observations	Basal Metabolic Rate	Average Cholesterol in Mg. Per 100 c.c. Blood	Method
Parenchymatous nephritis (nephrosis).	6	subnormal	510	Bloor modification of Funk-Autenreith method.
Chronic diffuse nephritis.	4	subnormal	300	
Myxedema.....	1	−19%	1350 (decreased to 206 under thyroid therapy)	Bloor modification of Funk-Autenreith method.
Myxedema.....	1	−14%	313	
Non-toxic adenoma of thyroid.	6	normal	176	Bloor modification of Funk-Autenreith method.
Menopause.....	10	normal	234	
Exophthalmic goiter.	21	+44%	144	Bloor modification of Funk-Autenreith method.
Toxic adenoma of thyroid.	10	+29%	182	

Since a large reserve of cholesterol is present in the cells of the body and since practically all staple foods contain it in varying amounts, it is not likely that a marked deficit occurs in the human body except under conditions of starvation, in the presence of wasting diseases such as carcinoma and Addison's disease, or the fatal cases of exophthalmic goiter.

The diet most suitable for patients with excessive amounts of blood cholesterol but with normal metabolic rates, should consist of fruit, vegetables, and milk, eliminating largely those foods which are known to contain high cholesterol values, such as eggs, meats, and fish, since with normal metabolic rates there probably will be no deficiency in protein utilization. An excess of carbohydrates in the diet calling for excess work on the part of the pancreas may under certain conditions prevent proper esterification of the blood cholesterol and its elimination,

with resulting concentration of cholesterol in the blood. This was observed by Luden in her experimental oatmeal diet.

For patients, however, who manifest disturbances associated with subnormal metabolic rates and increased blood cholesterol, the combination of high protein diet and thyroid therapy should be tried on the basis that the possible benefit from the administration of thyroid stimulates the rate of oxidation and promotes the assimilation of protein, the utilization of which is impaired. As mentioned by Epstein and Lande, the effect of thyroid in promoting utilization of protein may explain its influence on edema, especially in parenchymatous nephritis.

CHAPTER VIII

Total Nitrogen Determination in the Urine

(Modified from the Method of Folin)

1. With a measuring pipette place 0.2 c.c. filtered urine and 0.5 c.c. of distilled water in a Pyrex ignition test tube. With an ordinary pipette add 0.5 c.c. of the phosphoric-sulphuric-acid digestion mixture, made as follows:

Acid Phosphoric-Sulphuric Digestion Mixture

Copper sulphate solution (5 per cent)	50.0 c.c.
Acid phosphoric (85 per cent)	300.0 c.c.
Acid sulphuric, c. p. (free from ammonia)..	100.0 c.c.
Distilled water	450.0 c.c.

This should be kept well stoppered to prevent absorption of ammonia from the air. (Same as Solution 1 for blood non-protein nitrogen determination.)

2. Heat slowly over a micro-burner until the water is driven off and the color changes to dark brown, then cover tube with watch glass and continue heating gently until dense fumes fill the tube. Continue boiling at such a rate that the tube contains fumes but almost no fumes escape. The color should become clear or bluish-green after partially cooling. A drop of hydrogen peroxide may be added to clear the solution while the tube is warm. Remove flame and let cool for about two minutes. Rinse the tube thoroughly (the contents may be turbid from silica) with a little distilled water into a 25 c.c. volumetric flask. Shake to mix.

3. Transfer with a measuring pipette 1 c.c. of standard nitrogen solution (used in the determination of non-protein nitrogen in the blood, and which contains 0.2 mg. N per c.c.) into a 50 c.c. volumetric flask, add 1 c.c. of the phosphoric-sulphuric acid mixture to balance the acid in the unknown,

add about 10 c.c. of distilled water and shake to mix. The standard so prepared contains 0.2 mg. nitrogen.

4. When ready give each flask a whirl and add about 10 c.c. of Nessler's reagent to the unknown, and about 20 c.c. to the standard. When full development of color has been secured add sufficient distilled water to bring volume in the unknown to the 25 c.c. mark and in the standard to the 50 c.c. mark.

5. If the unknown Nesslerized mixture is turbid from silica, centrifuge a portion before the color comparison is made. The white sediment in the tube is silica. *If the sediment is deeply colored the Nesslerization was not successful and should be discarded.* Wait five minutes for color to develop before comparing in the colorimeter.

6. With standard set at 20 mm., with R indicating the reading of the unknown solution, the calculation will be as follows

$$\frac{20}{R} \times \frac{0.2 \text{ mg.}}{2} = \text{mg. N in 0.2 c.c. urine,}$$

or

$$\frac{1000}{R} = \text{mg. N in 100 c.c. urine.}$$

7. Example: If the twenty-four hour quantity of urine equaled 1560 c.c. and the reading of the unknown was 15, the result would be $\frac{1000}{15} \times 15.6 = 1040$ mg. or 1.04 gm.

Titratable Acidity of Urine, in Terms of N/10 NaOH (Method of Folin)

1. Place 10 c.c. of urine in a flask, add about 6 gm. of finely pulverized neutral potassium oxalate,[1] and 1 to 2 drops of 1 per cent phenolphthalein solution as indicator.

2. Shake solution vigorously one to two minutes and titrate with N/10 NaOH until the solution turns a faint pink which remains permanent when the solution is shaken.

3. If 1200 c.c. represents the twenty-four hour volume of urine, and 6 represents the number of cubic centimeters of N/10 NaOH used, the total acidity will be calculated as follows:

[1] Potassium oxalate is added to precipitate the calcium which would interfere with the titration end-point when the urine is neutralized.

10:6::1200:x or, $10x = 7200$ or, $x = 720$, the acidity of the twenty-four hour urine in terms of cubic centimeters of N/10 NaOH.

The acidity may also be represented in terms of percentage. In the example above the acidity per 100 c.c. in terms of N/10 NaOH would be 60.

Phenolsulphonephthalein Determination of Kidney Function

1. Have patient empty bladder and then drink 300–400 c.c. of water.

2. Twenty minutes later inject 1 c.c. (from ampule containing 6 mg.) of phenolsulphonephthalein into gluteal or lumbar muscles.

3. One hour and ten minutes later have patient empty bladder (ten minutes of this time interval is to allow the dye to reach the kidneys). Add 200 c.c. water to urine and 1 c.c. of 10 per cent sodium hydroxide solution to bring out the deep purple color, then dilute to 1000 c.c. Label "first-hour" specimen.

4. One hour later have patient empty bladder. Add 200 c.c. of water to the urine and 1 c.c. of 10 per cent sodium hydroxide solution, then dilute to 1000 c.c. Label "second-hour" specimen.

5. The standard solution required contains 3 mg. phenolsulphonephthalein and 1 c.c. of 10 per cent sodium hydroxide solution and volume made to 1000 c.c.

6. Set standard in colorimeter at 10 and make comparison with first- and second-hour specimens. R equals reading of unknown. The calculation will be $\frac{10}{R} \times 50$ or $\frac{500}{R}$ = per cent of dye excreted.

7. The first-hour specimen should equal 40–60 per cent; the second-hour specimen 20–25 per cent more, or a total in the two-hour period of 60–85 per cent. *An average of many so-called normal estimations has equaled 48 per cent for the first hour and 17 per cent for the second hour, or a total of 65 per cent.*

CHAPTER IX

Clinical Comments on the Diagnosis of Impaired Kidney Function

Degenerative processes affecting the cardiovascular renal systems, produced by many processes, including acute and chronic infections as well as the wear and tear incidental to life, are such important factors leading to death before the expiration of the normal life expectancy, and constitute such an important part of the work of the physician in his endeavor to prevent the development of such lesions, that an understanding of this phase of clinical medicine is essential in the care of practically every patient who has reached the fifth decade of life.

The endeavor to determine what factors are concerned in producing evidence of early damage to these organs involves in practically every case knowledge of the blood chemistry as well as an investigation of kidney function in a more comprehensive manner than such tests are usually performed. A little time and a comparatively small outlay spent in equipping a laboratory, as well as training a young woman in the essentials of the work, will make available for every physician the information desired. The reward in added satisfaction that the advice given is founded upon fact, rather than upon the basis of a casual examination of a single urinary specimen, will be many times worth the time consumed and the effort used. Among the essential facts to be determined in an examination of kidney function are the following:

The Urine Examination

1. In the absence of edema the quantity of urine voided varies normally with the intake of fluid. Variations in humidity, the amount of physical exercise, and the occurrence of diarrhea may be mentioned as factors disturbing the normal ratio of

intake to output. Normally the quantity of urine voided during the twelve-hour night period should not exceed 50 per cent of the quantity voided during the twelve-hour day period.

2. The specific gravity should vary between 1.010 and 1.030, depending upon the amount of fluid taken, the humidity and other factors, such as exercise and nervous or emotional stress. Normally there should be evident no tendency toward so-called fixation of specific gravity toward either the higher or the lower points in a series of specimens passed at different periods during the twenty-four hours.

3. All specimens should normally be free from abnormal ingredients such as albumin, sugar, diacetic acid, casts, blood-cells and pus. A few leucocytes in specimens from patients who have suffered a previous inflammatory or traumatic lesion in any portion of the genitourinary tract may be found for long periods subsequently. Specimens from women who have borne children and who have relaxed bladder walls normally contain a considerable number of leucocytes. The finding of an occasional hyaline cast in a centrifugated specimen, especially from an individual near the middle point of life, may be ignored.

4. The urea content of any specimen should be 2 per cent or higher. The hypobromite method for urea is sufficiently accurate for clinical purposes. If the patient has been under supervision with weighed diets so that the total intake of protein in grams is known (from which the nitrogen intake can be computed by dividing by 6.25) it aids in an understanding of the patient's condition if the "nitrogen balance" is determined by a computation of the total nitrogen eliminated in the twenty-four hour specimen of urine.

5. The output of sodium chloride in any specimen varies according to intake from 1 to 2.5 per cent or higher. The Volhardt method is satisfactory. A modification of this method may be performed as follows: Dilute 10 c.c. of urine with 90 c.c. of water to which should be added 1 or 2 drops of 25 per cent nitric acid. The mixture should then be made alkaline with 10 per cent solution of sodium carbonate. A few drops of

a 10 per cent solution of potassium chromate solution are added as an indicator. Titration is then performed with N/10 silver chloride solution. Each cubic centimeter of the silver solution used equals 0.00583 gm. of sodium chloride.

6. The output of phthalein as a measure of the excretory function of the kidneys should under normal conditions approximate 40 to 60 per cent during the first hour, and 20 to 25 per cent during the second hour, or a total of 60 to 85 per cent during the first two hours.

7. The usual tests for acetone bodies in the urine are, as Folin has shown, tests for diacetic acid. The sodium nitroprusside test is more delicate than the ferric chloride reaction. These tests, when persistently positive, indicate disturbed metabolism dependent usually upon dehydration and so-called "acid poisoning." The administration of salicylates and coal-tar products such as phenacetine may produce a diacetic reaction in the urine.

The Blood Examination

1. The test diet of Mosenthal designed to measure the excretory capacity of the kidneys has served a useful purpose in calling attention to phases of the subject not generally appreciated. For its proper interpretation most careful attention to detail is necessary, conditions being obtained, as a rule, only with the cooperation of a trained dietician during the period of observation. Under this plan blood chemistry studies and deductions incidental to the test diet and measured fluid intake are correlated with examinations of the measured urinary specimens, all of which has for its purpose the determination of kidney function under more or less artificial conditions. There are many reasons for believing, as Mosenthal has recently stated,[1] that if the various phases of renal function are studied while the patient is following his usual daily routine, a more valuable estimate of how he should adjust his habits to the conditions found to exist may be obtained than by carrying out arti-

[1] Mosenthal, Herman O. Value of tests for renal function in clinical medicine. *Ohio M. J.*, Columbus, May, 1922.

ficial dietetic tests that impose standards which may not be applicable or approximate the normal daily routine of the individual. It is obvious that in carrying out any investigation the ordeal must be simplified as much as possible for the patient. For ambulatory patients the following plan is recommended: For the twenty-four hours preceding the time set for taking the sample of blood before breakfast, the patient collects the urine specimens as follows: Upon arising the bladder is emptied. This urine is discarded. All specimens passed from 8 a.m. to and including 8 p.m. are measured and a portion of each placed in bottles which are to be brought to the laboratory. The urine passed after 8 p.m. to 8 a.m., including the amount voided upon arising, is collected in one container, measured, and a portion labeled "8 p.m. to 8 a.m." is brought to the laboratory. The patient is instructed to drink no fluid after the evening meal. If possible a record should be kept of the amounts of fluid taken during the day. For many patients it is only necessary to collect and measure the urine passed during the day and night twelve-hour periods, a portion of each being brought for examination. The data accumulated in such an examination may conveniently be summarized in the form of a chart which includes the blood chemistry findings.

		Day quantity After 8 a.m.–8 p.m.	Night quantity After 8 p.m.–8 a.m.
Urine Specimens *Quantity c.c.*			
Specific gravity of different specimens.			
Greatest variation in specific gravity	=		
Total nitrogen output 24-hour quantity	=		
Total sodium chloride 24-hour quantity	=		
Abnormal ingredients, Albumin	=		
Sugar	=		
Diacetic acid	=		
Chlorides	=		
Microscopic			
Phthalein output, first hour	=	per cent; second hour =	per cent
Fasting blood chemistry		mg. per 100 c.c. blood.	
Non-protein nitrogen	=		
Urea	=		
Preformed creatinin	=		
Uric acid	=		
Sugar	=		
Chlorides	=		
Cholesterol	=		

CHAPTER X

The Dietary Control of Disturbances of Metabolism

I. Neutral, Alkali and Acid-Producing Foods

The following lists of foods useful in the treatment of certain disturbed metabolic states are appended. They are based upon the ash analyses of Sherman and Gettler and have been tested on man by Blatherwick.The first three have been published by Sansum of the Potter Metabolic Clinic.

1. *Neutral Foods*

Butter	Cornstarch
Cream	Sugar
Lard	Tapioca

2. *Alkali-Producing Foods*

In the following list the excess of base or alkali over acid is expressed in terms of cubic centimeters of a normal solution.

	Per 100 Gm.
FRUITS	
Raisins	23.63
Muskmelons	7.47
Pears, dried	7.07
Oranges	6.61
Currants	5.97
Bananas	5.56
Lemons	5.45
Peaches	5.04
Apples	3.76
VEGETABLES	
Beans, lima, dried	41.65
Beans, dried	23.87
Beets	10.86
Carrots	10.82
Celery	7.78
Lettuce	7.37

VEGETABLES (Cont.)	*Per 100 Gm.*
Potatoes	7.19
Cauliflower	5.33
Cabbage	4.34
Radishes	2.87
Turnips	2.68
Asparagus	0.81
NUTS	
Almonds	12.38
Chestnuts	7.42
MISCELLANEOUS	
Cow's milk	2.37

3. *Acid-Producing Foods*

In the following list the total excess of acidity over base is expressed in terms of cubic centimeters of a normal solution. The ash of these foods is alkaline in reaction, but since they contain benzoic acid, which is changed to hippuric acid before elimination, the body acidity is increased when these foods preponderate in the diet.

MEATS AND FISH	*Per 100 Gm.*
Oysters	30.0
Chicken	17.0
Haddock	16.0
Rabbit	14.8
Beef, lean	13.9
Veal	13.5
Pike	11.8
Pork, lean	11.8
Frog	10.3
EGGS	
Yolk	26.6
Eggs, whole	11.1
CEREALS	
Oatmeal	12.9
Rice	8.1
Crackers	7.8
Corn, sweet, dried	5.9
Bread, whole wheat	3.0
Bread, white	2.7

NUTS

	Per. 100 Gm
Peanuts	3.9

FRUITS

Cranberries	
Prunes	
Plums	

For reference the following table has also been found useful. In it the excess of base or alkali over acid in 100 calories of each food is given:

Spinach	113.0	Buttermilk	6.1
Cucumbers	45.5	Squash	6.1
Celery	41.1	Apples (fresh)	6.0
Chard	41.1	Pumpkin	5.7
Lettuce	38.6	Bananas	5.6
Rhubarb	37.0	Pears (fresh)	5.6
Figs	32.3	Potatoes (sweet)	5.4
Tomatoes (canned)	24.5	Milk (skimmed)	5.0
Carrots	24.0	Beans (baked)	5.0
Beets (fresh)	23.6	Grape-juice	4.0
Molasses	20.0	Potatoes (chips)	3.9
Muskmelons	19.0	Cranberries	3.7
Olives	18.8	Asparagus	3.6
Parsnips	18.2	Chestnuts	3.2
Cabbage	18.0	Dates	3.2
Cauliflower	17.4	Onions	3.1
Pineapple	15.7	Citron	3.0
Orange-juice	14.4	Koumiss	2.9
Beans (string)	13.0	Grapes	2.8
Raspberry juice	13.0	Milk (condens. unsw.)	2.7
Peaches (fresh)	12.2	Milk (whole)	2.6
Lemons	12.0	Beans (kidney)	2.5
Oranges	11.0	Pears (canned)	2.3
Lemon-juice	11.0	Almonds	1.8
Apricots	11.0	Currants	1.8
Peaches (canned)	10.0	Peas (canned)	1.5
Radishes	9.8	Peas (dried)	1.5
Mushrooms	8.9	Milk (condens. sweet)	1.4
Watermelon	8.8	Cocoanuts	1.2
Potatoes (white)	8.6	Peas (green)	1.2
Prunes	8.0	Cream (18% fat)	0.3
Cherries	7.8	Cream (40% fat)	0.1
Plums	7.3	Marmalade	0.1
Turnips	7.0	Cocoa	0.1
Raisins	6.8		

II. Minimum Salt and Basic Alkali Diet

For general purposes the following list of foods has been useful in the treatment of edema in chronic nephritis of the types associated with hyperarterial tension. In this list the content of salt is small, the protein restricted, and an excess of alkali-producing foods is present in the amounts ordinarily consumed. The amount of fluid intake should be restricted to 700–1000 c.c. daily. The following foods are allowed: Bread or toast (made without salt), sugar, farina, custard, fresh butter, rice, tea, milk (250 c.c. daily), baked apples or apple sauce, orange-juice, melons, grape-fruit, lettuce, jello, bananas, prunes. All other green vegetables may be allowed but should be boiled in two waters to remove the salt.

III. Clinical Comments

The accumulated experience of many physicians has shown that patients who have disturbed kidney function, definite nephritis, hyperarterial tension, rheumatic symptoms (when due to improper diet), headaches due to overfeeding, lack of exercise and improper elimination, obscure neuralgic and neuritis-like pains (not due to foci of chronic infection), and other similar conditions, are generally benefited by restriction of the meats and eggs. This benefit aside from the restricted albumin intake may with much reason be ascribed, especially in most typical types of chronic nephritis and the associated acidosis, to the influence of diminished acid formation, the deleterious effect of which upon the kidneys has been thoroughly investigated by Martin Fischer. The only apparent exceptions to such a regime as regards the protein intake are to be found in the treatment of certain types of chronic nephritis in which subnormal basal metabolism may be present, and in which it may be believed that impaired utilization of protein exists. This has been discussed under blood cholesterol.

It will be noted from the above list that certain cereals are acid-producing foods. From a practical standpoint, if the diet is largely made up of the base or alkali-producing foods given

above, the moderate consumption of breadstuffs will not greatly influence the result desired.

In general, with the exception of cranberries, prunes and plums, all fruits, vegetables, and nuts (with the exception of peanuts) are basic (alkaline) in nature. The following foods, among those mentioned in the list above, have been found useful clinically in reducing the body acidity as measured by the reaction of the urine: Melons, apples, oranges, bananas, lemons, carrots, beets, lettuce, celery and potatoes. The fruit acids are largely converted into alkali carbonates in the intestine, hence they may be considered alkaline in nature. The neutral foods do not increase the body acidity and may be used in ordinary amounts unless special reason for restriction, such as acidosis, exists, in which case the intake of fats, such as cream, butter, and lard, should be limited. For ordinary clinical control I have found it useful to give to the patient or nurse a supply of methyl-red papers, after a suggestion by Martin Fischer, by means of which the reaction of the urine may be tested once or twice daily.

IV. The Purin Constituents of Foods

Since it appears highly desirable to limit the intake of purin-containing foods in the treatment of conditions due to abnormal retention of uric acid in the blood, the following table of Walker's, quoted in the Presbyterian Hospital (New York) Diet Lists, is added:

I

Meats	*Purin bodies, gm. per kilo.*
Beef	1.3–2.0
Pork	1.2
Chicken	1.2
Ham	1.1
Veal	1.1
Salmon	1.1
Halibut	1.0
Mutton	0.96
Cod	0.5
Meat soups	(varying large amounts)

Vegetables	*Purin bodies, gm. per kilo.*
Beans, kidney	0.63
Oatmeal	0.53
Peas	0.39
Lentils	0.38
Asparagus	0.21

Drinks	*Per 500 c.c.*
Coffee	1.7
Tea	1.2
Cocoa	1.0
Chocolate	0.7

The foods listed in the above table should be limited in conditions associated with uric acid retention. In addition to those mentioned, the glandular meats, such as sweetbreads, liver, kidney, and brain; whole-wheat products, such as graham or whole-wheat bread or shredded wheat; and malt preparations, such as beer, ale or porter, should be restricted.

The following foods are purin-free or contain a negligible amount:[1]

Cereals
Rice
Hominy
Farina
Cream of Wheat

Breadstuffs
Flour (white)
Bread (white)
Corn meal
Spaghetti
Macaroni
Biscuits (white)
Crackers (white)

Dairy Products
Butter
Milk
Buttermilk
Cream

Vegetables
Potatoes (Irish or sweet)
Cauliflower
Onions
Cabbage
Lettuce
Eggplant
Spinach
Brussels sprouts
Corn

Desserts
Nuts
Cheese (American, Swiss, and Cream)
Ice cream and ices
Cake (except coffee or chocolate)
Puddings (bread, tapioca, or cornstarch)
Pie (apple, custard, or cocoanut)

Miscellaneous
Jam and marmalade
Sugar and syrup
Fresh and cooked fruits
Bacon
Soups (cream or vegetable)
Eggs

[1] From Von Noorden & the Vanderbilt Clinic Diet Lists.

Beverages
Carbonated water
Vichy
Grape-juice
Loganberry-juice
Cider
Malted milk

The following "moderate protein-low purin" diet, consisting largely of neutral and alkali-producing foods has been found useful in the treatment of nephritis. It contains approximately 2000 calories, an amount sufficient to maintain efficiency for the ambulatory patient who is obliged to do a moderate amount of work. For obese patients a diet may be appropriately followed which consists largely of fruits and vegetables.

MODERATE PROTEIN-LOW PURIN NEPHRITIC DIET

	Wt. in gm. or c.c.	Protein	Fat	Carbohydrate	Calories	Remarks
Breakfast						
1 egg	...	6.2	5.6	....	76.0	
Farina (cooked)	100	1.7	0.2	11.5	56.0	If 100 gm. hominy are used prot. = 2.2; fat = 0.2; carbo. 17.8; calories 84. If 100 gm. boiled rice, prot. 2.8; fat 0.1; carbo. 24.4; calories 112
Sugar	15	....	.....	15.0	60.0	
Cream (20 per cent)	90	3.0	18.0	3.0	180.0	Usual market cream
Toast (white bread 2 slices)	60	5.5	0.8	32.0	160.0	
Butter	15	0.15	12.7	....	119.0	If edema is present use fresh butter
Orange or peach marmalade (1 hp. tablespoonful)	30	0.18	.....	25.3	105.0	
Luncheon						
Cream vegetable soup	250	6.4	18.0	14.6	242.0	Made without meat stock from corn, potatoes, asparagus, celery, cauliflower, thickened with cornstarch
Butter	15	0.15	12.7	....	119.0	
Bread (white 2 slices)	60	5.5	0.8	32.0	160.0	If edema is present use bread made without salt

Moderate Protein-Low Purin Nephritic Diet

Dinner						
Vegetables (cooked).....	60	1.0	.....	2.0	12.0	Vegetables with 3 per cent available carbohydrate used, which include the following: Asparagus, cauliflower, Brussels sprouts, beet greens, cabbage, rhubarb, spinach, string beans, eggplant. If vegetables with 6 per cent available carbohydrate are used, such as the following: Onions, squash, turnips, carrots, mushrooms, or beets, add 1.0 gm. protein and 2.0 gm. carbohydrate to intake and 12 calories
Potato (baked) med. size....	130	3.7	0.2	32.0	145.0	
Milk (glass).....	180	6.0	7.2	9.0	130.0	
Head lettuce (½ head).....	50	0.6	0.15	1.5	10.0	
French dressing (1 tbsp.)....	22	....	16.0	....	150.0	Made in proportion 4 tbsp. olive or other vegetable oil, 1 tbsp. lemon juice, ¼ teasp. salt
Bread (white 1 slice).....	30	2.75	0.4	16.0	80.0	
Butter.....	15	0.15	12.7	....	119.0	
Baked apple.....	120	0.6	0.6	30.0	128.0	3 heap. tbsp. apple sauce contains prot. 0.25; fat 1.0; carbo. 46, calories 194
Totals.....	...	43.5	106.0	224.0	2051.0	

CHAPTER XI

The Test and Maintenance Diets of Joslin in the Treatment of Diabetes Mellitus

So much improvement in the management of diabetic patients has followed the teachings of Joslin that a brief résume of his plan of treatment is appended. The following test diets were designed by him for use by the patient to reduce gradually the amount of sugar present in the urine until the urine is sugar-free, and to decrease the amount of sugar present in the blood. When this has occurred the Maintenance Diets are begun, to establish tolerance and a sugar-free state. As Joslin has stated, this plan is an arbitrary one and the majority of cases will require some modification of it. Much more can be accomplished, however, by following such an established guiding principle than by attempting to secure the result desired by less laborious short-cut methods, which in the end react little to the benefit of the patient or the credit of the physician.

The Test Diets are to be used for a period of five days during which the patient gradually becomes sugar-free. On successive days advances are to be made from Test Diet No. 1 to Test Diet No. 5. If on the fifth day the urine still contains sugar, fasting can be employed for one or more days. During the fast days clear broths, water, clear coffee, tea, and whiskey or brandy may be allowed.

The Maintenance Diets are to be begun as soon as the patient's urine does not contain sugar. If this should occur on the fifth day while the patient is taking Test Diet No. 5 he may begin the next day on Maintenance Diet No. 1. The plan will then carry him through on successive days the use of Maintenance Diets 2, 3, 4, etc. until sugar appears in the urine. Certain patients can progress steadily day by day until Maintenance Diet No. 10 or No. 12 is taken without showing sugar. If sugar should appear in the urine, for example, while the patient is taking Maintenance Diet No. 6, one should drop back

in the amount of carbohydrate allowed to that contained in the diet two days earlier, i.e., to Maintenance Diet No. 4, while still continuing the same amounts of protein and fat allowed in Diet No. 6. Such a diet should perhaps be continued for two or three days or until the urine is again sugar-free. The patient's approximate carbohydrate tolerance may then be assumed to have been established. The tolerance may not always be maintained at this point; for a variety of conditions, such as an acute infection, may upset it. The next step should be to increase the amount of protein and fat allowed as follows: If the patient can take the amount of carbohydrate allowed in Maintenance Diet No. 4 and the amount of protein and fat allowed in No. 6, the attempt should be made to increase the calories of protein and fat until he can take the amounts allowed in Diet No. 7 or Diet No. 8, the aim being to furnish a total of 52 to 30 calories for each kilogram (2.2 lbs.) of patient's body weight, which amount is sufficient for maintenance "at rest."

If in carrying out the Test Diets it should be found that the patient becomes sugar-free on Diet No. 3, it will not be necessary to begin with Maintenance Diet No. 1, but instead one may begin with Maintenance Diet No. 6, which contains an amount of carbohydrate about equal to that contained in the Test Diet upon which the patient became sugar-free.

Experience with the recently discovered substance insulin, which has been found of such great value in making it possible for the diabetic patient to utilize more carbohydrate, will modify these dietary suggestions according to the need of the patient and the judgment of the physician.

Approximate Weight Equivalents*
(Used in following tables)

			C.	P.	F.
5 per cent vegetables	300 gm.	= Three moderate portions	10.0	5.0	0.0
Orange	300 gm.	= One and one-half large size	26.0	1.8	0.0
Skimmed milk	480 c.c.	= One pint	25.7	17.2	1.5
Fish (halibut, mackerel)	120 gm.	= Two small portions	0.0	24.0	4.8
Potato	240 gm.	= Two medium size	48.0	8.0	0.0
Meat (cooked, lean)	90 gm.	= One moderate portion	0.0	24.0	15.0
Bread	90 gm.	= Three small slices	54.0	9.0	0.0
Oatmeal (dry weight)	30 gm.	= One large saucerful	20.0	5.0	2.0
Cream "average"	60 c.c.	= Three tablespoonfuls	2.1	2.25	12.0
Bacon	30 gm.	= Four crisp strips	0.0	5.0	15.0
Butter	30 gm.	= Three medium portions	0.0	0.0	25.0
Cottage cheese	60 gm.	= Four level tablespoonfuls	2.0	12.0	0.0
Egg (one)			0.0	6.0	6.0
Shredded wheat biscuit	30 gm.	= One biscuit	22.6	3.0	0.4
Uneeda crackers	12 gm.	= Two crackers	8.7	1.2	1.1

The total sugar-forming constituents of a diet should be computed as 100 per cent of the carbohydrate, 58 per cent of the protein and 10 per cent of the fat intake. The fat used should not exceed twice the carbohydrate plus one-half the protein (Woodyatt formula).

1 gm. carbohydrate = 4 calories.
1 gm. protein = 4 calories.
1 gm. fat = 9 calories.

* The computations are figured from the publications of Joslin and from Locke's "Food Values."

Test Diets

Test diets	Weight in grams	Vegetables, 5 per cent	Orange	Oatmeal*	Shredded wheat	Uneeda	Potato	Bread	Egg	Cream, 20 per cent	Bacon	Butter	Meat	Fish	Skimmed milk
No. 1	Carbo..... 189 Protein..... 89 Fat..... 15 Calories.....1247	300	300		1		240	90					90	120	480
No. 2	Carbo..... 102 Protein..... 58 Fat..... 0 Calories..... 640	300	300		1		120							180	300
No. 3	Carbo..... 64 Protein..... 33 Fat..... 0 Calories 388	300	300				60							90	240
No. 4	Carbo..... 36 Protein..... 27 Fat..... 0 Calories..... 252	300	200											90	120
No. 5	Carbo..... 15 Protein..... 5 Fat..... 0 Calories..... 80	300	50												

* Oatmeal always measured by dry weight.

Maintenance Diets

Maintenance diets	Weight in grams	Carbohydrate							Protein and Fat						
		Vegetables, 5 per cent	Orange	Oatmeal*	Shredded wheat	Uneeda	Potato	Bread	Egg	Cream, 20 per cent	Bacon	Butter	Meat	Fish	Skimmed milk
No. 1	Carbo..... 10 Protein..... 11 Fat..... 6 Calories..... 138	300							1						
No. 2	Carbo..... 22 Protein..... 13 Fat..... 18 Calories..... 302	300	100						1	60					
No. 3	Carbo..... 32 Protein..... 24 Fat..... 24 Calories..... 440	600	100						2	60					
No. 4	Carbo..... 42 Protein..... 29 Fat..... 39 Calories..... 635	600	200						2	60	30				
No. 5	Carbo..... 52 Protein..... 32 Fat..... 53 Calories..... 813	600	200	15					2	60	30	15			

*Oatmeal always measured by dry weight.

MAINTENANCE DIETS (*Continued*)

Maintenance diets	Weight in grams	Carbohydrate							Protein and Fat						
		Vegetables, 5 per cent	Orange	Oatmeal*	Shredded wheat	Uneeda	Potato	Bread	Egg	Cream, 20 per cent	Bacon	Butter	Meat	Fish	Skimmed milk
No. 6	Carbo..... 63 Protein..... 43 Fat..... 65 Calories.....1009	600	200	30					2	90	30	15	30		
No. 7	Carbo..... 73 Protein..... 51 Fat..... 70 Calories.....1126	600	300	30					2	90	30	15	60		
No. 8	Carbo..... 83 Protein..... 60 Fat..... 88 Calories.....1364	600	300	30		2			2	90	30	30	90		
No. 9	Carbo..... 96 Protein..... 63 Fat..... 94 Calories.....1482	600	300	30	½	2			2	120	30	30	90		
No. 10	Carbo..... 107 Protein..... 64 Fat..... 94 Calories.....1530	600	300	30	1	2			2	120	30	30	90		

*Oatmeal always measured by dry weight.

MAINTENANCE DIETS (*Continued*)

Maintenance diets	Weight in grams	Carbohydrate							Protein and Fat						
		Vegetables, 5 per cent	Orange	Oatmeal*	Shredded wheat	Uneeda	Potato,	Bread	Egg	Cream, 20 per cent	Bacon	Butter	Meat	Fish	Skimmed milk
No. 11	Carbo..... 131 Protein..... 76 Fat..... 99 Calories.....1719	600	300	30	1	2	120		2	120	30	30	120		
No. 12	Carbo..... 155 Protein..... 80 Fat..... 99 Calories.....1831	600	300	30	1	2	240		2	120	30	30	120		

*Oatmeal always measured by dry weight.

List of 5 Per Cent Vegetables* and Fruits

Lettuce
Cucumbers
Spinach
Asparagus
Rhubarb
Endive
Marrow
Sorrel
Sauerkraut
Beet greens
Dandelion greens
Swiss chard
Celery
Mushrooms
Tomatoes
Brussels sprouts
Water cress
Sea kale
Okra
Cauliflower
Egg plant
Cabbage
Radishes
Leeks
String beans, canned
Broccoli
Artichokes, canned
Ripe olives
Grape fruit

List of 10 Per Cent Vegetables* and Fruits

String Beans
Pumpkin
Turnip
Kohl-rabi
Squash
Beets
Carrots
Onions
Green peas, canned
Watermelon
Strawberries
Lemons
Cranberries
Peaches
Pineapple
Blackberries
Gooseberries
Oranges

List of 15 Per Cent Vegetables* and Fruits

Green peas
Artichokes
Parsnips
Lima beans, canned
Raspberries
Currants
Apricots
Pears
Apples
Huckleberries
Blueberries
Cherries

List of 20 Per Cent Vegetables* and Fruits

Potatoes
Shell beans
Baked beans
Green corn
Boiled rice
Boiled macaroni
Plums
Bananas
Prunes

* Fresh or canned.

Clinical Comments on Diabetic Management

The discovery of insulin has made it no longer necessary to maintain diabetic patients in a state of undernutrition. In beginning treatment it has not been found wise to depend too much upon household or approximate measurements. It will be found much more satisfactory to use a food scale.[1]

In the treatment of patients with severe diabetes, especially those who manifest evidences of more or less disturbed kidney permeability, many difficulties may arise. In such patients it may be difficult to decrease the blood sugar to near the normal amount, although sugar may not be constantly present in the urine because of the higher renal threshold for sugar elimination. If the endeavor is made to restrict the carbohydrates for many such patients to a minimum, serious symptoms of acidosis may arise. The most important of such symptoms are: The presence of the so-called acetone odor to the breath, the presence of diacetic acid in the urine, the presence of hyperpnea or of nausea or vomiting, and an early tendency to sluggish speech or sleepiness. A high protein, high fat and low carbohydrate diet is a combination upon which many such patients do not do well. Patients with marked arteriosclerosis with or without hypertension will usually do better and be in safer condition if the protein is more or less restricted and the carbohydrates moderately increased, especially if the blood sugar is not high and sugar is present in the urine in only small amounts. For many patients of this type it has been found necessary in order to render them sugar-free to proceed very slowly in the restriction of carbohydrates. If the intake is very gradually restricted until the urine is sugar-free and then the tolerance is slowly built up it may be possible to keep the urine free from sugar for long periods without fasting. Should symptoms of acidosis arise, the fats should be restricted and the patient saturated with water. The attempt should be made to give one liter of water every four hours by mouth or by proctoclysis.

[1] A 500 gm. accurate food scale is recommended.

The injection of Ringer's solution into the vein is also a useful method. This should be given slowly.

It is most important that diabetic patients, when ill, even from trivial causes, should go to bed and take a glass of hot water, tea, clear coffee, broth, orange-juice, or oatmeal water gruel every hour. The bowels should be emptied by an enema. The possibility of an impending coma should always be borne in mind.

Should coma develop, insulin, if available, should be used at the earliest possible moment. The dosage will depend upon the clinical condition of the patient, which includes, if time allows, the blood-sugar percentage. In general, 10 units may be given every hour for two or three doses, then, if necessary, every two or three hours for two or three doses more. Experience has indicated that not more than 60 to 100 units should be given during the first twelve hours. In addition a 5 per cent glucose and 2 per cent soda bicarbonate solution should be given by the drip method per rectum, while glucose and orange-juice or coffee may be given by mouth or by gavage. In order to prevent dehydration, fluids should be forced either by gavage or by hypodermoclysis of normal salt solution.

When insulin becomes more available for general use, the matter of proper dosage of this valuable substance will be better understood. Experience has so far indicated that it is much safer to begin treatment with the small dosage of one unit, increasing the dose daily as rapidly as possible. One unit of insulin will enable the patient to utilize approximately from 2.5 to 4.0 gm. additional carbohydrate.

Should too large a dose of insulin be given with resulting rapid reduction in blood sugar, symptoms of nervousness, weakness, hunger, increased pulse rate, and sweating may occur. These symptoms are relieved by giving sugar in some form, by the hypodermic injection of ten to fifteen minims of 1–1000 solution of adrenalin chloride, or, as Banting has recently reported, by the intravenous injection of calcium chloride. Banting has also reported that calcium lactate in 10-gr. doses

may be given to children three times daily to prevent the development of shock during insulin treatment.

In case of contemplated operation upon a diabetic patient, nitrous oxide gas-oxygen should be the anesthetic of choice. As a general rule ether anesthesia is unsafe. It may be considered a safe rule before any operation upon a diabetic patient is performed, whether of major or minor degree, to restrict the intake of fats for a few days regardless of his apparent tolerance and freedom from symptoms of acidosis. Nearly every physician has seen serious sequelæ follow minor operations, such as may have been performed by chiropodists, leading to gangrene because of failure to consider the possibility of an impending acidosis.

After operation upon a diabetic patient it usually is not wise, at least for the first few days, to give anything more than plenty of water by mouth or proctoclysis, or Ringer's solution by vein, albumin water, orange-juice, fresh pineapple-juice, and oatmeal gruel. The 5 per cent vegetables are not as a rule well tolerated during this trying period because of the stomach disturbance which is apt to occur.

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